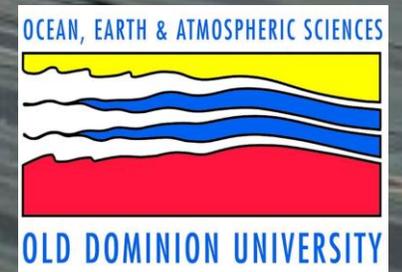


2020 Virginia HABs: Estuarine monitoring summary

Margie Mulholland
Old Dominion University
Department of Ocean and Earth Sciences

VA HAB Taskforce Meeting
Gloucester Point, Virginia
1/15/2021



Overview

- Bloom response - ODU
- Notable 2020 blooms
- Monitoring results summary
- Related projects –transport, IFCB
- 2021

VDH Shellfish monitoring

- Monthly collections- routine fixed sites
 - Lugol's solution (500mL) – phytoplankton analyses (ODU)
 - Unpreserved frozen sample (50mL)- ELISA screening (VDH)
- Bloom samples
 - Response to bloom reports or visual observation by field staff
 - VDH, CBP, HRSD, Time series site

National Shellfish Sanitation Program (NSSP)

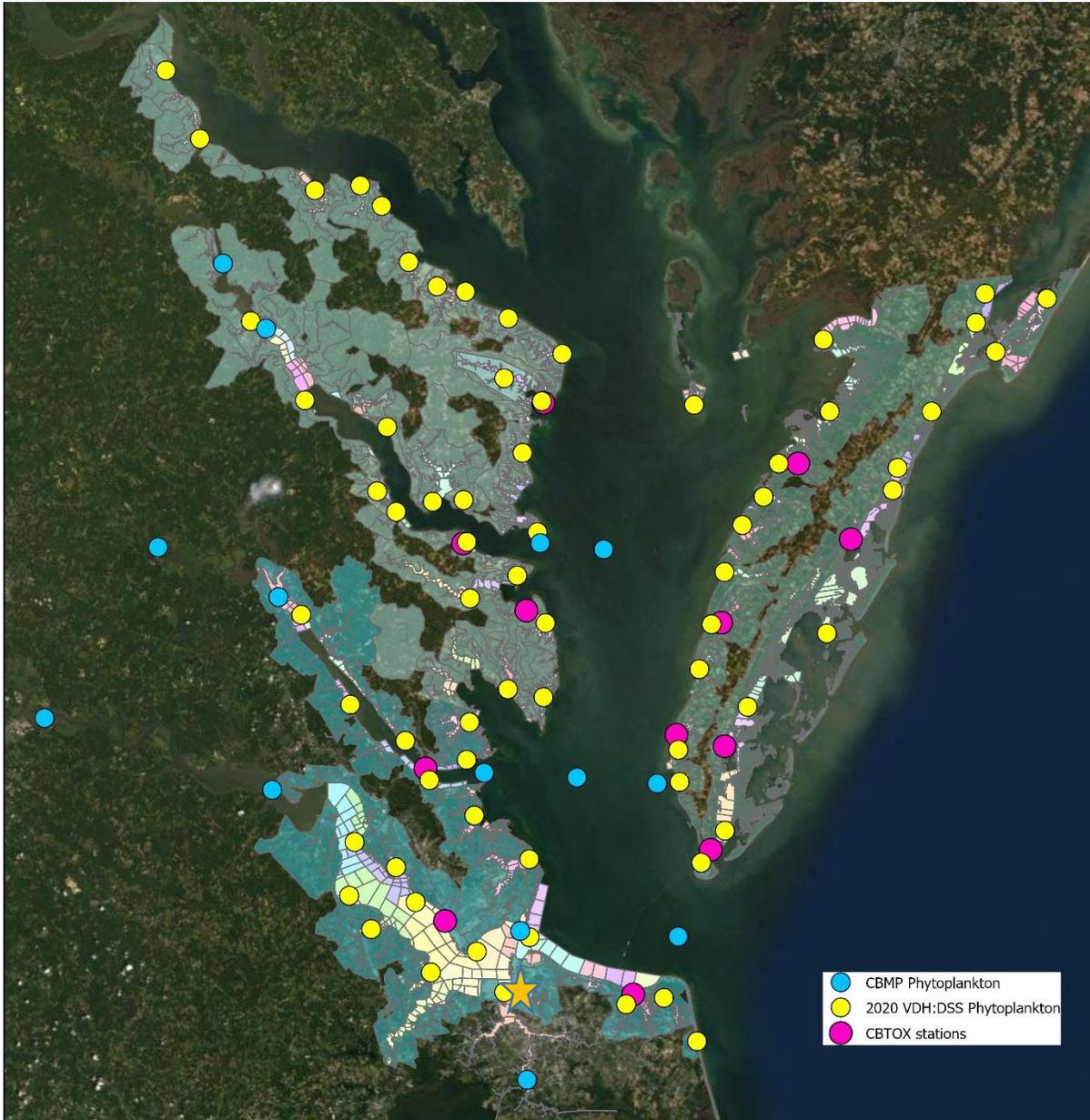
Guide for the Control of Molluscan Shellfish 2017 Revision



From the U.S. Food and Drug Administration website
<http://www.fda.gov/Food/GuidanceRegulation/FederalStateFoodPrograms/ucm2006754.htm>



Phyto Kit: Extra bottles, vials, lugol's, rubber gloves, marker



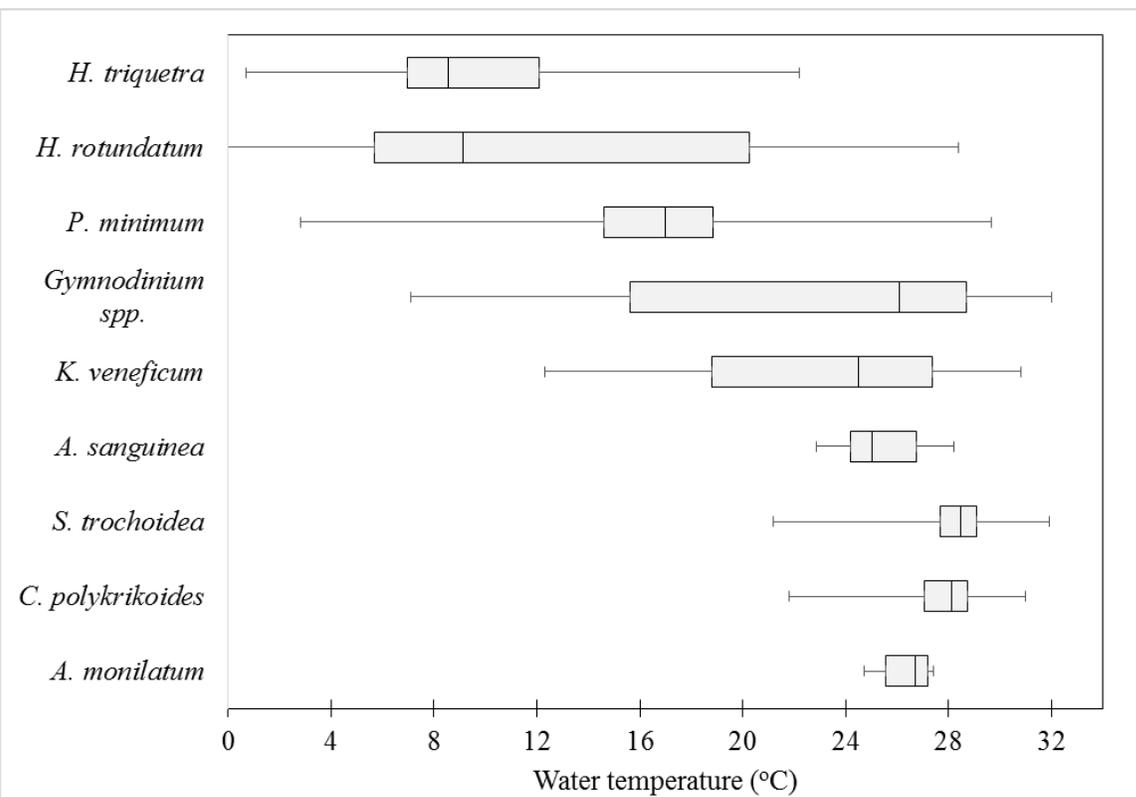
Virginia Estuarine Phytoplankton monitoring

- Chesapeake Bay Monitoring Program (DEQ/ODU)
 - 14 stations
 - 7-Chesapeake Bay monthly year-round
 - 7-Tidal tributaries monthly March-October
- Full species composition
- Ad hoc bloom sampling
- VDH: Shellfish (DSS&WHC/ODU)
 - 69 stations
 - Monthly year-round
 - Targeted HAB identification
 - Targeted toxin screening (based on cell counts)
- CBTOX (VDH:DSS/VIMS)
 - 12 stations (2017-2018)
 - 4 stations (2019-2020)
 - Bi-weekly sampling
 - Targeted HAB identification
 - Routine toxin analyses
- ★ Additional monitoring: ODU, HRSD, ECOHAB
Dataflow HRSD (no bloom samples in 2020)

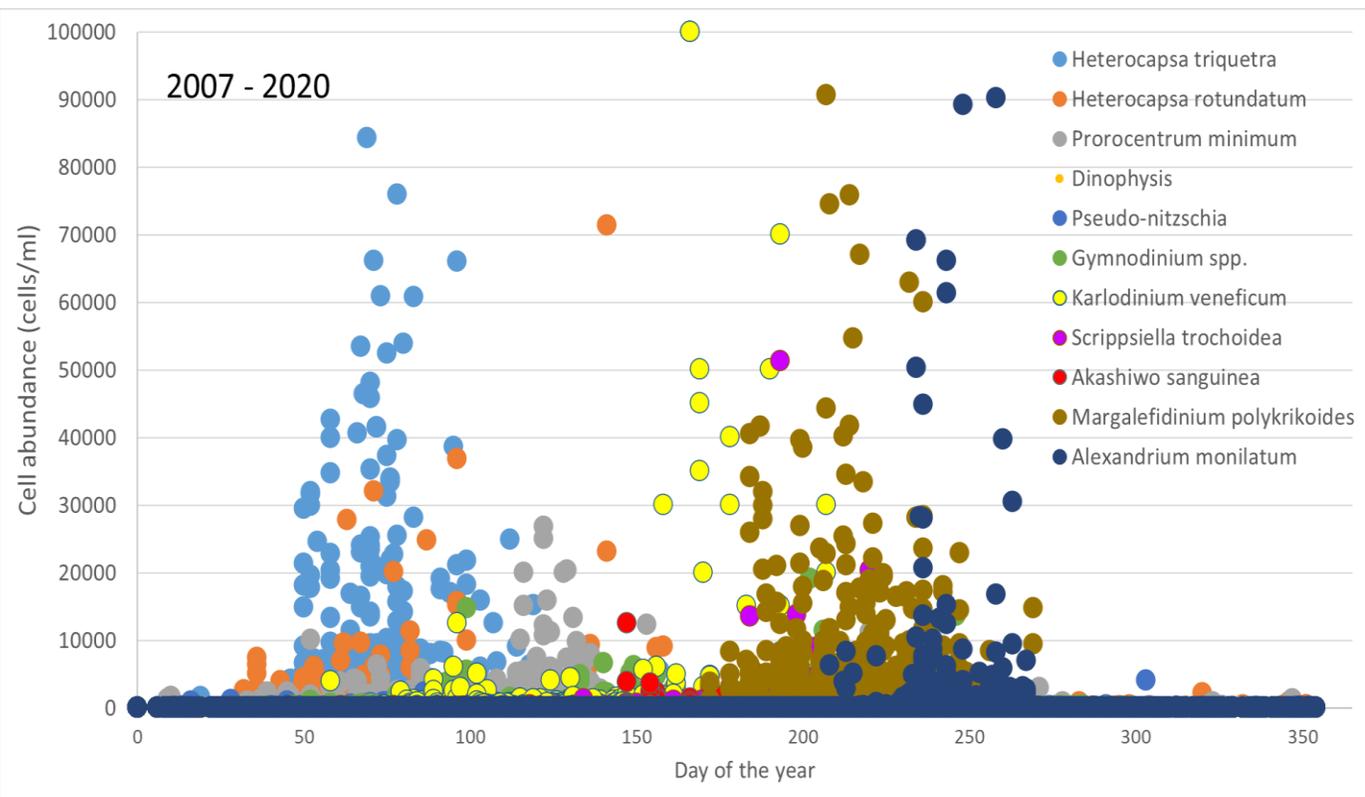
COVID gaps

- No samples from HRSD dataflow from James River so didn't have the data density of previous years
- No CBP sampling March-April; no trib sampling March-June
- No VDH sampling mid-March – mid-May
- Did do near-daily sampling in the Elizabeth and Lafayette Rivers throughout the year and were able to document *Heterocapsa* bloom there via IFCB
- Still need to count time series data from Lafayette River

“Normal” bloom progression



**Pseudo-nitzschia* and *Dinophysis* not abundant enough to make the list



*Removed data where abundances were > 100,000 cells/ml

Notable 2020 Blooms:

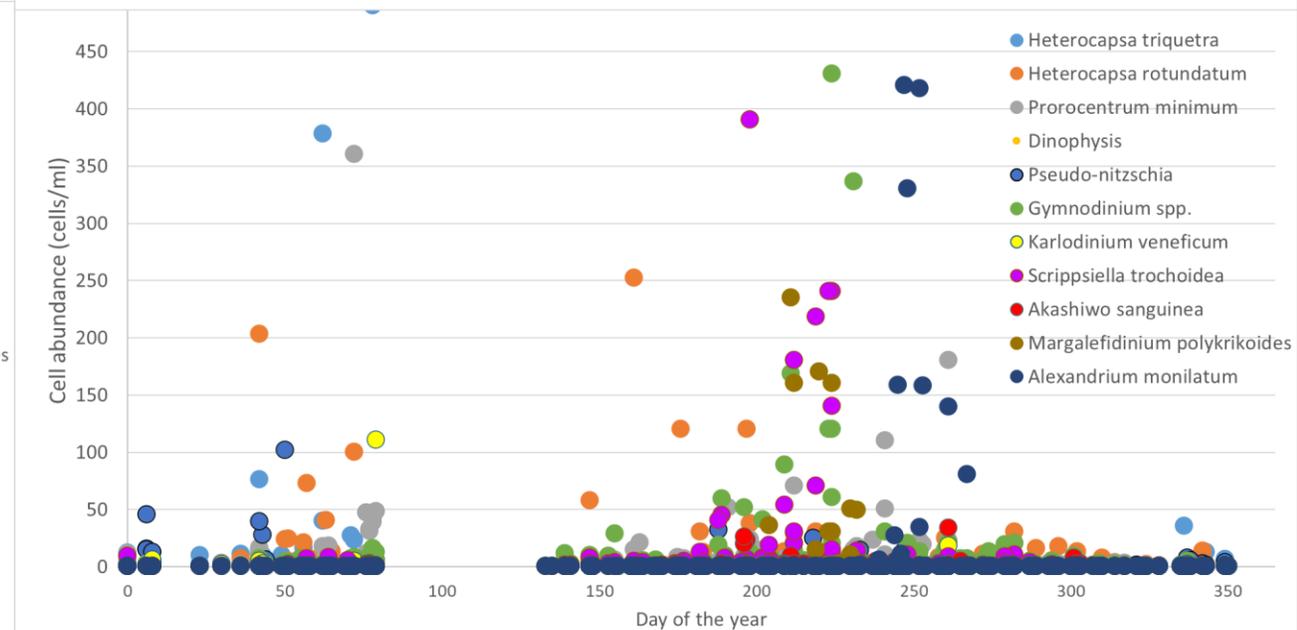
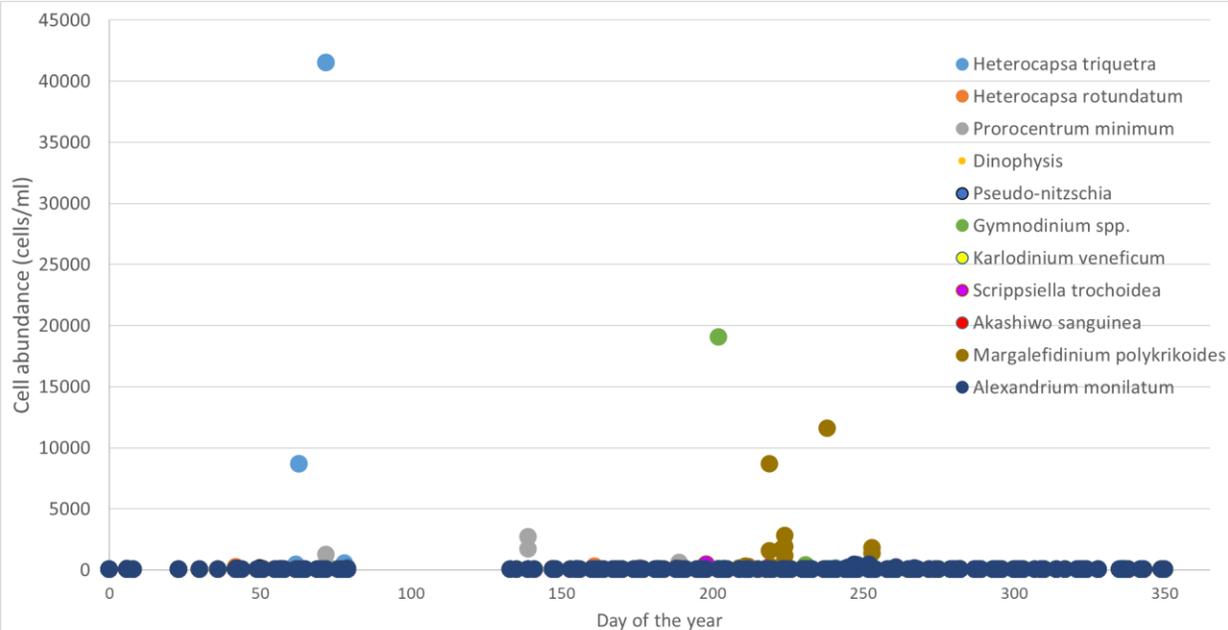
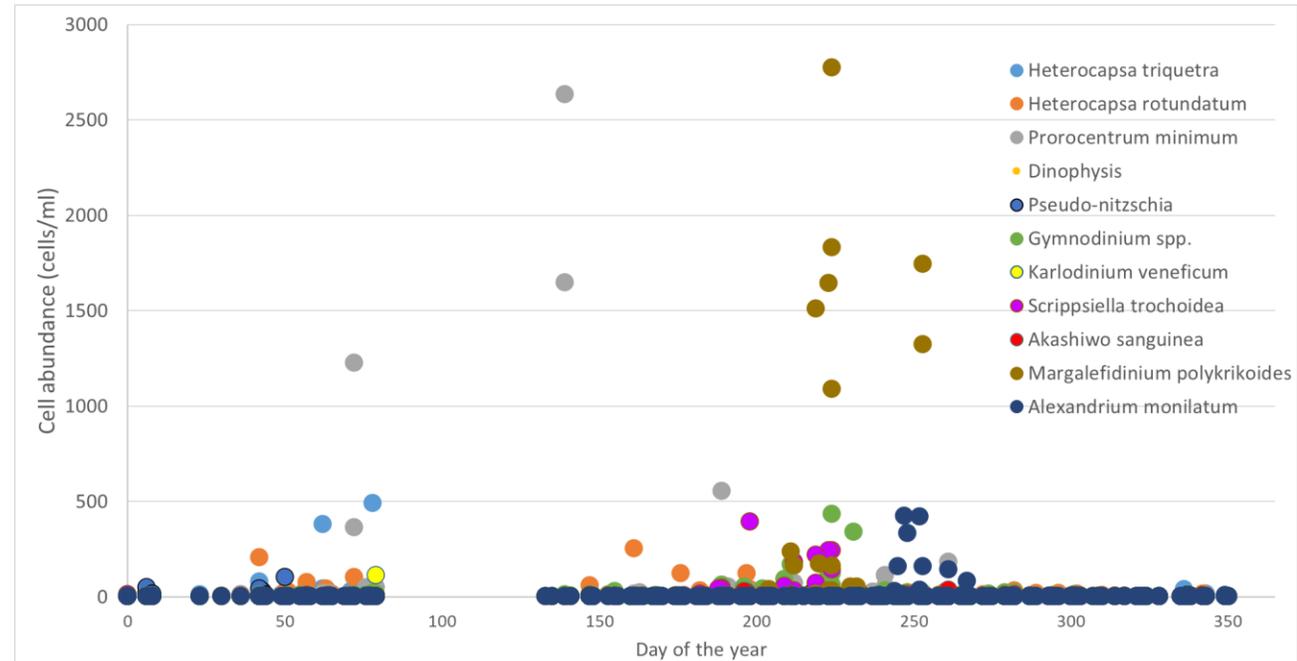
Heterocapsa triquetra (January -March)

Prorocentrum minimum (March – May)

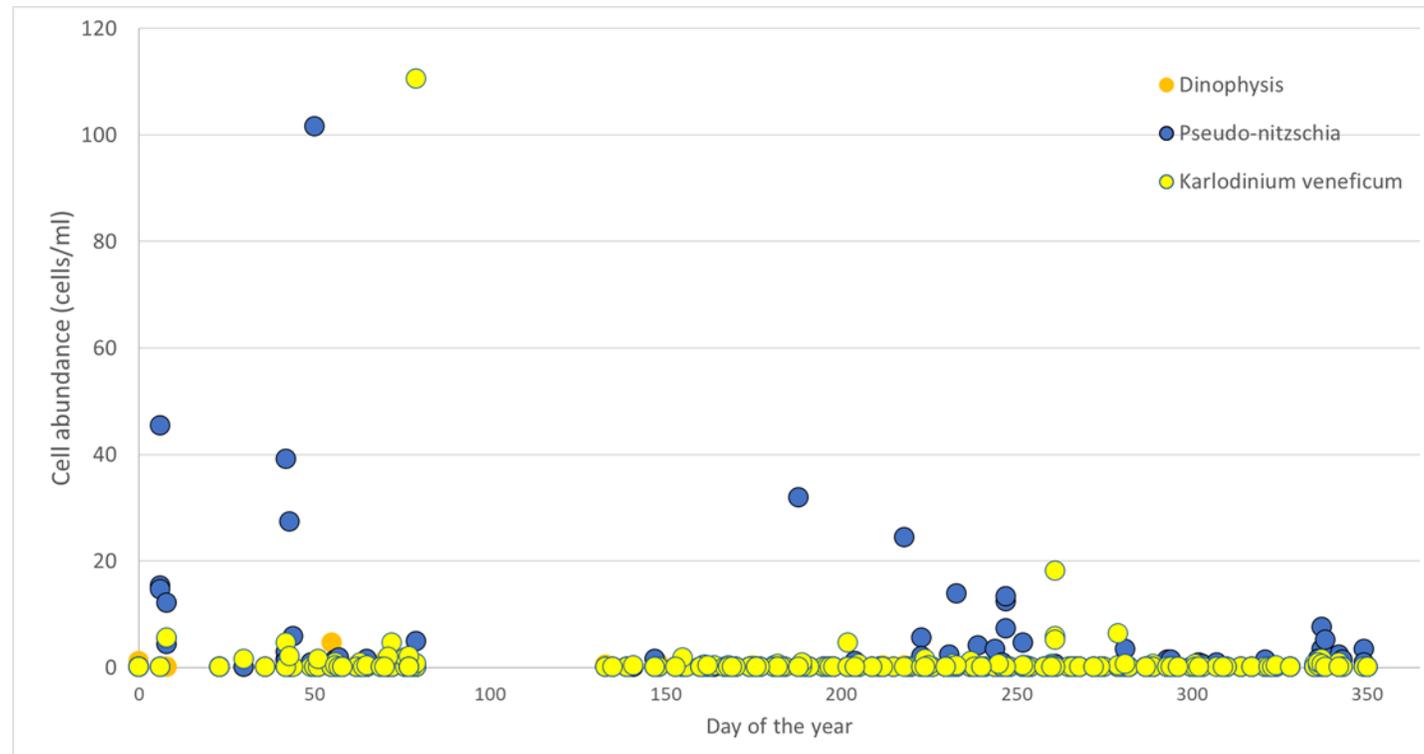
Margalefidinium polykrikoides (July – August)

Alexandrium monilatum (August – September)

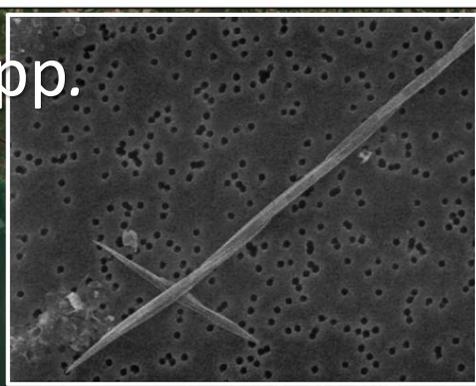
But had the full complement of species.



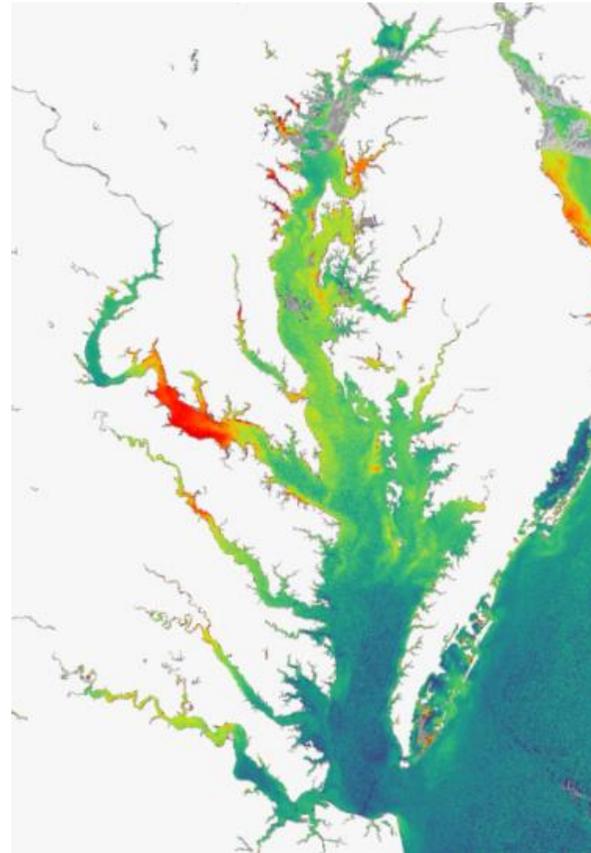
Our less abundant HAB species in 2020



Pseudo-nitzschia spp.



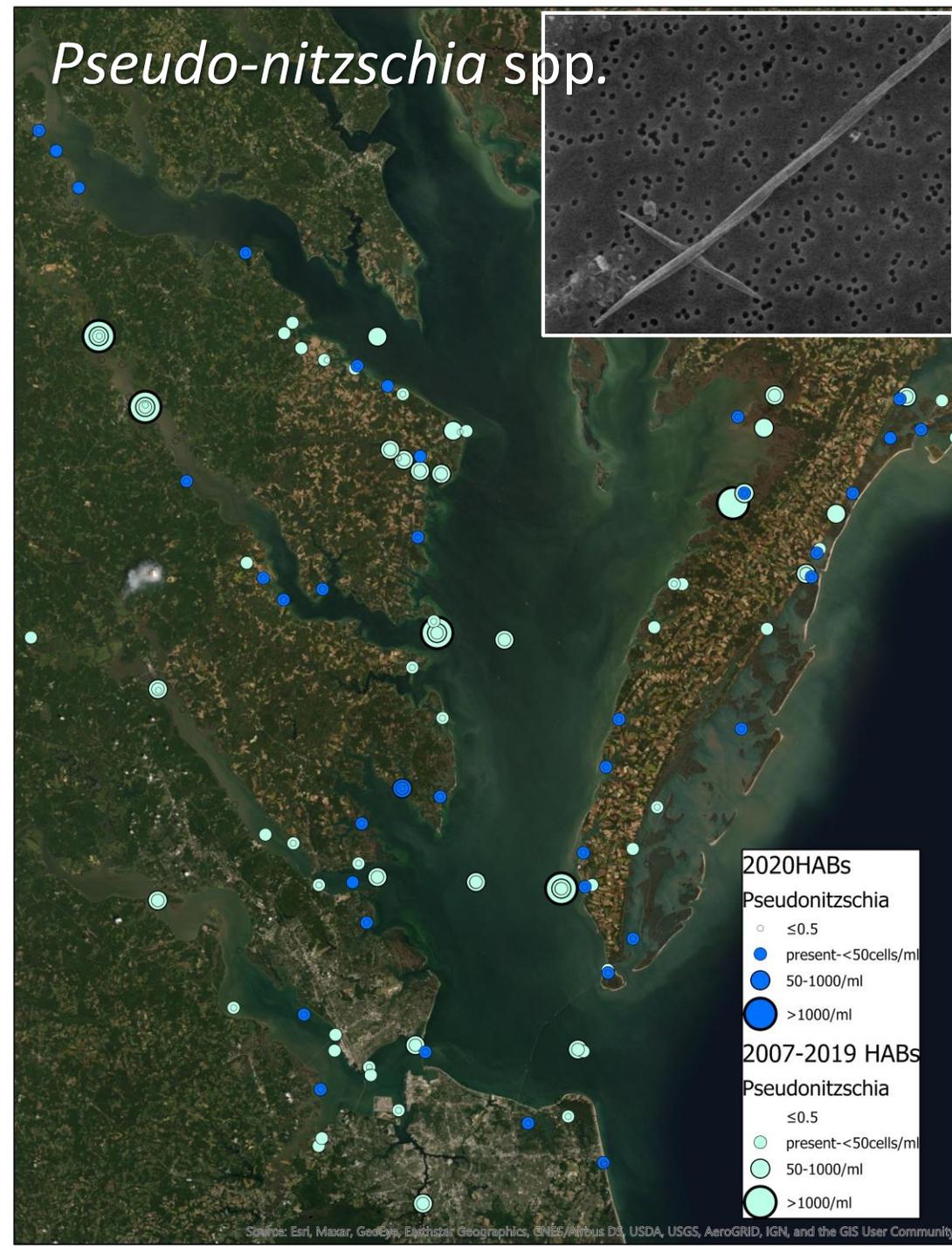
- January 2020 Potomac *Pseudo-nitzschia* event - Mixed diatom bloom-*Skeletonema* dominant



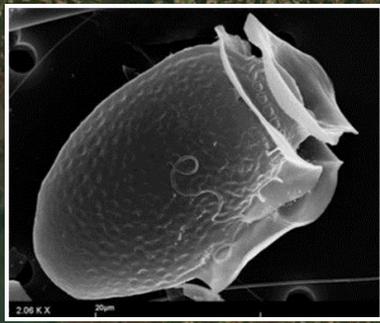
- MD DNR/MDE results ~800- >1000 cells/ml
- Special sample collections DSS: January 6, 2020
- *Pseudo-nitzschia* densities 52-73 cells/ml
- Toxin BDL

OAA: MODIS-Sentinel imagery-1/1/2020

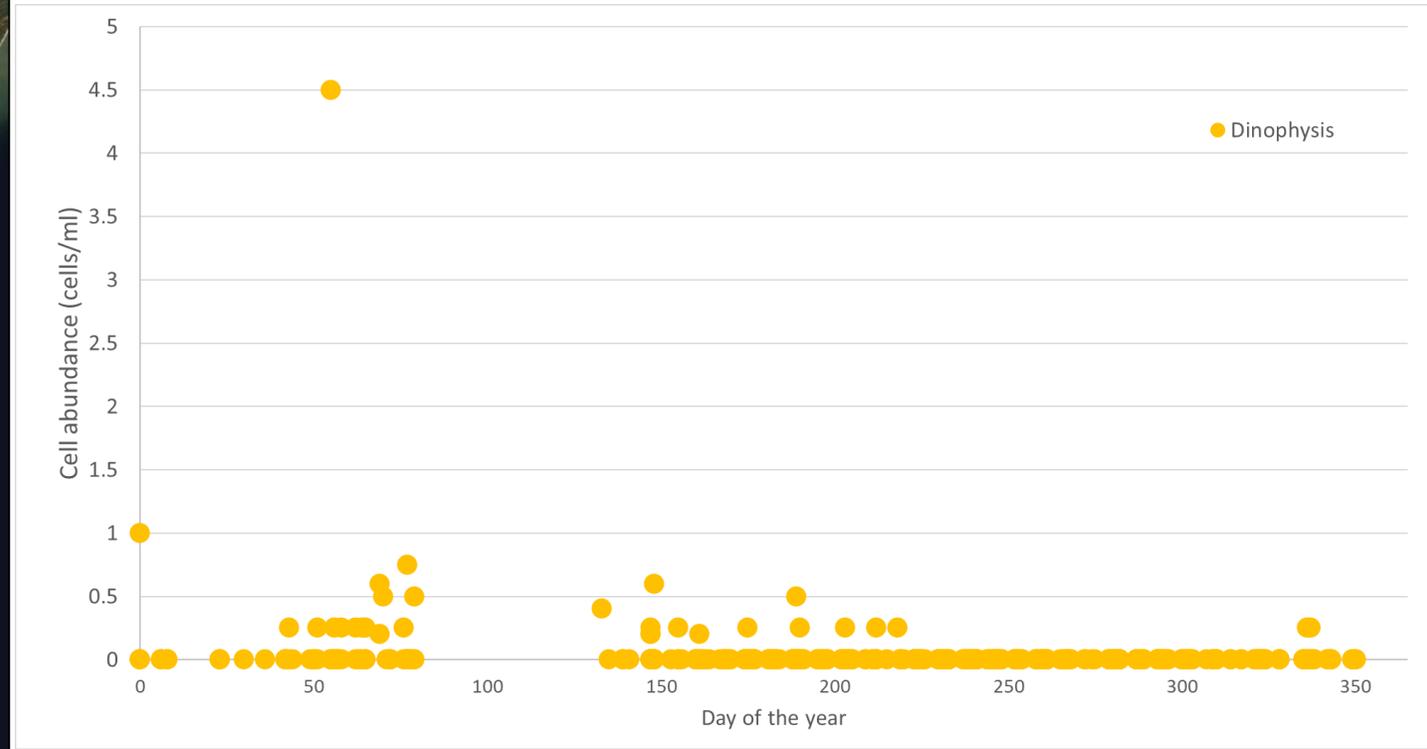
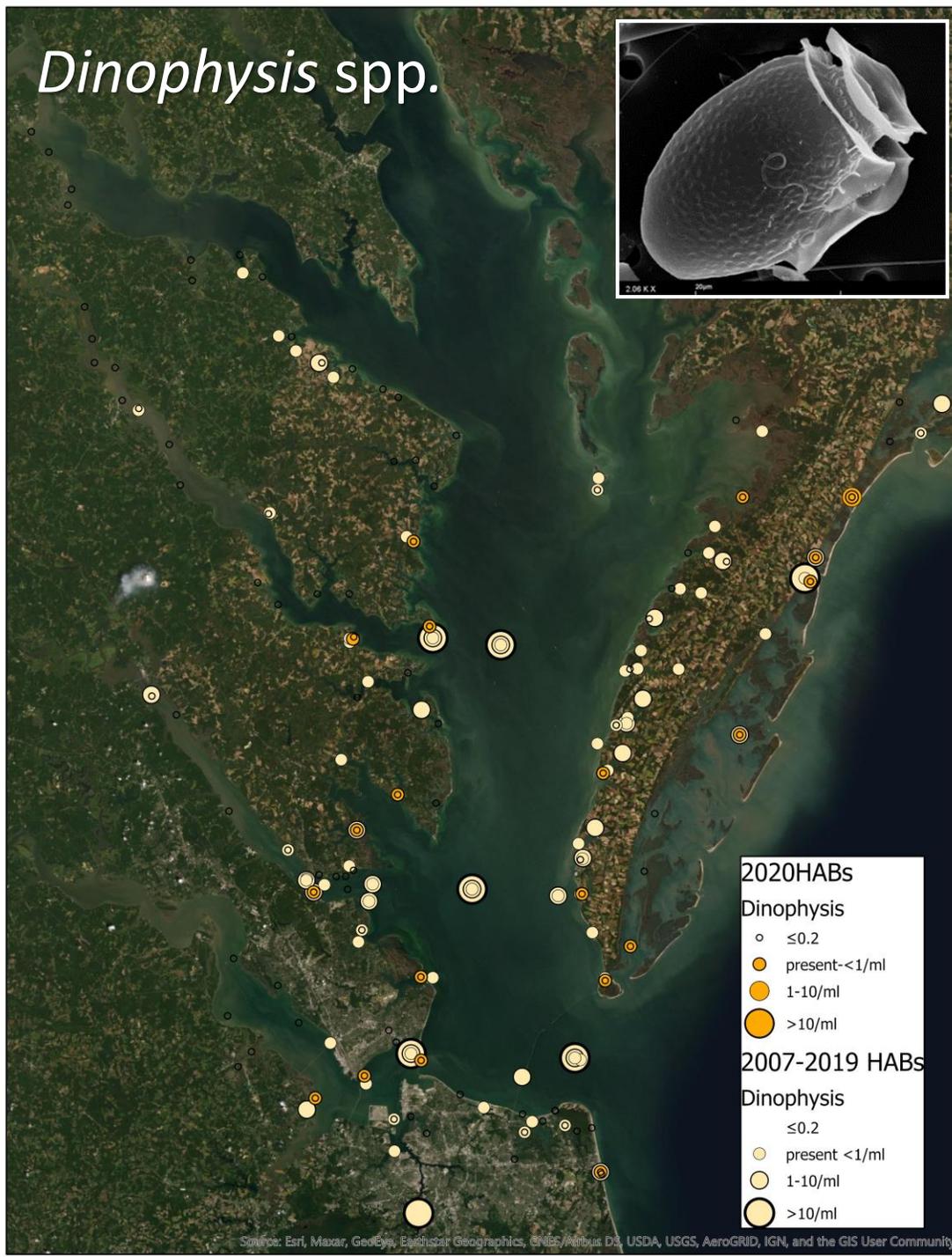
- Generally low cell densities
- Widespread distribution in Chesapeake Bay and seaside Eastern Shore



Dinophysis spp.

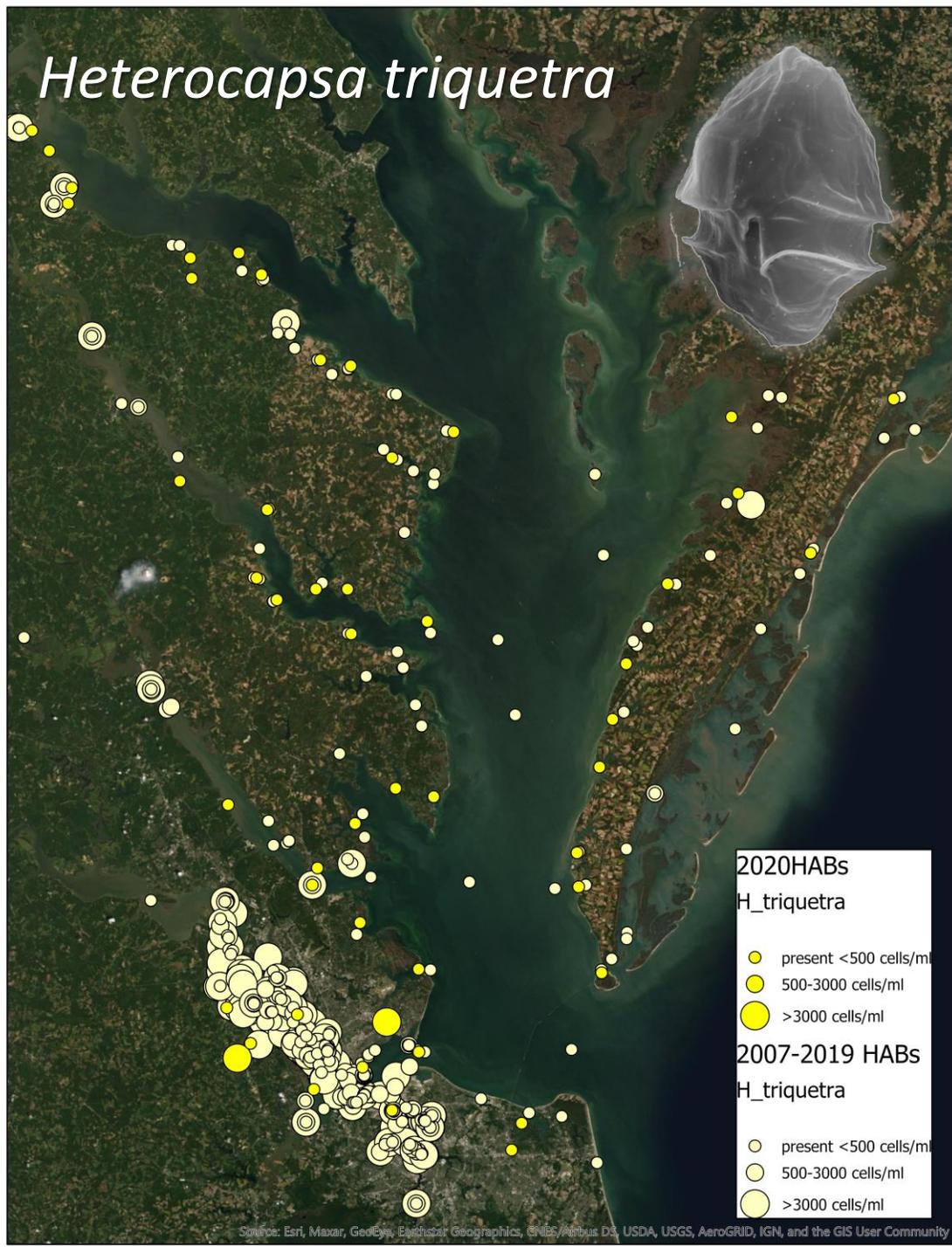


- Generally low cell densities
- Widespread distribution in Chesapeake Bay and seaside Eastern Shore

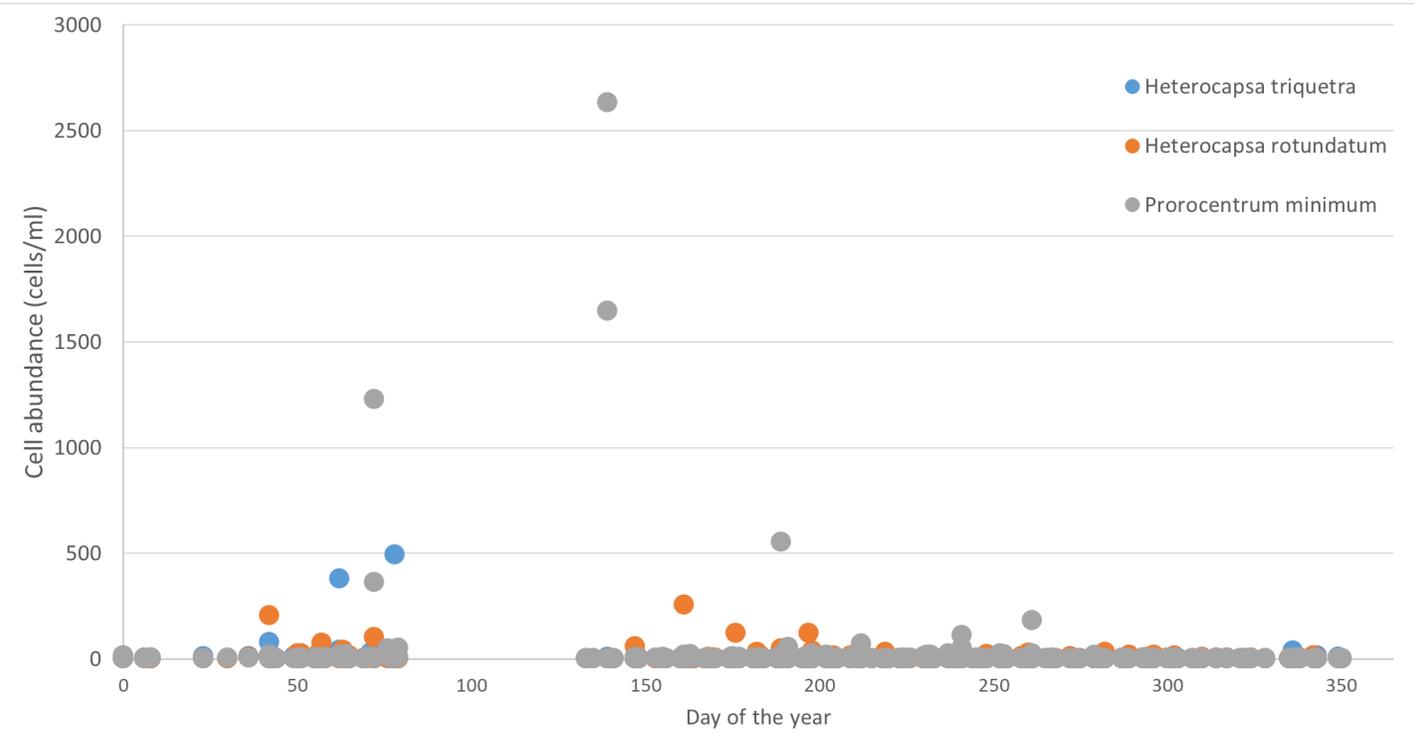


Source: Esri, Maxar, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

Heterocapsa triquetra

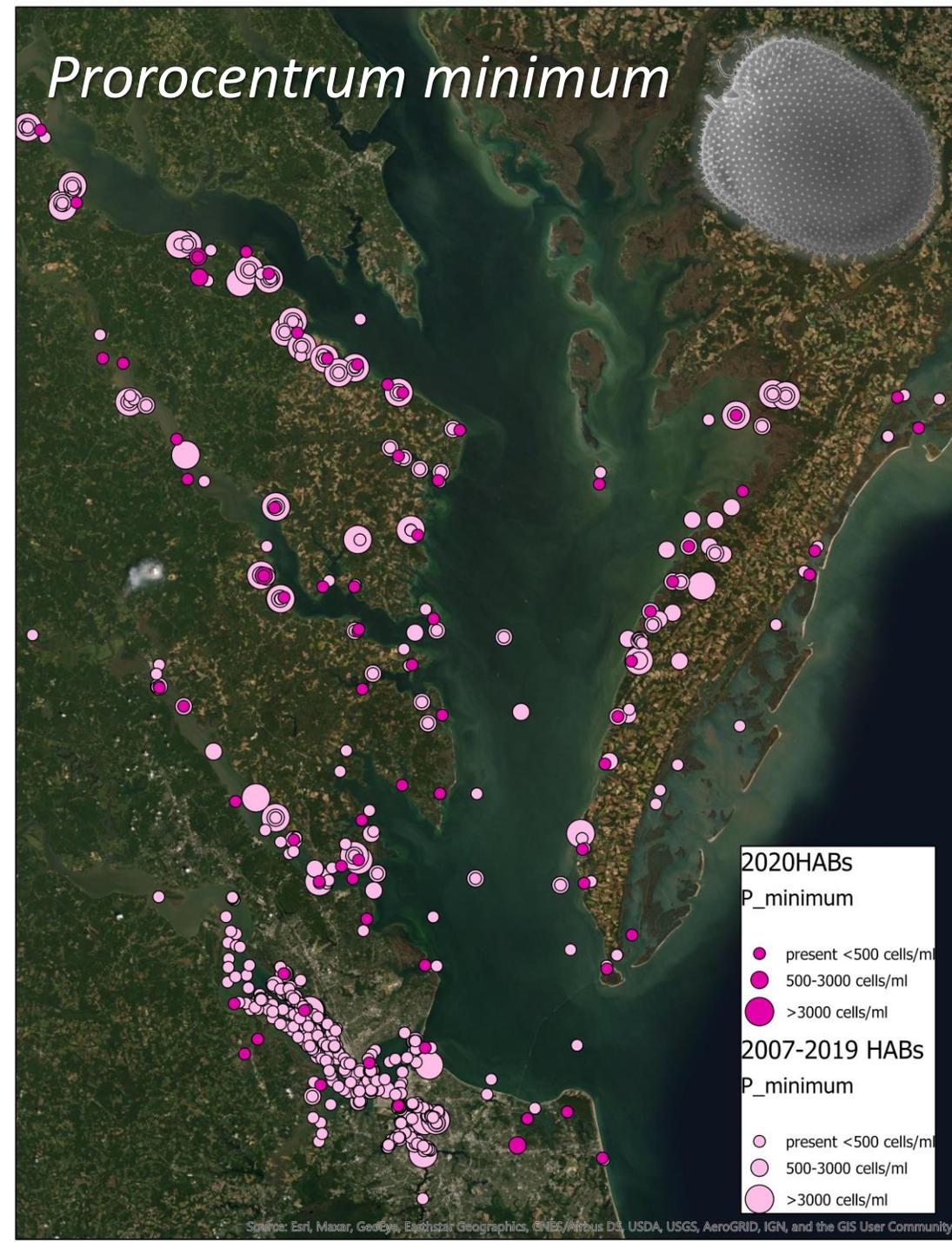


Bloom January - March

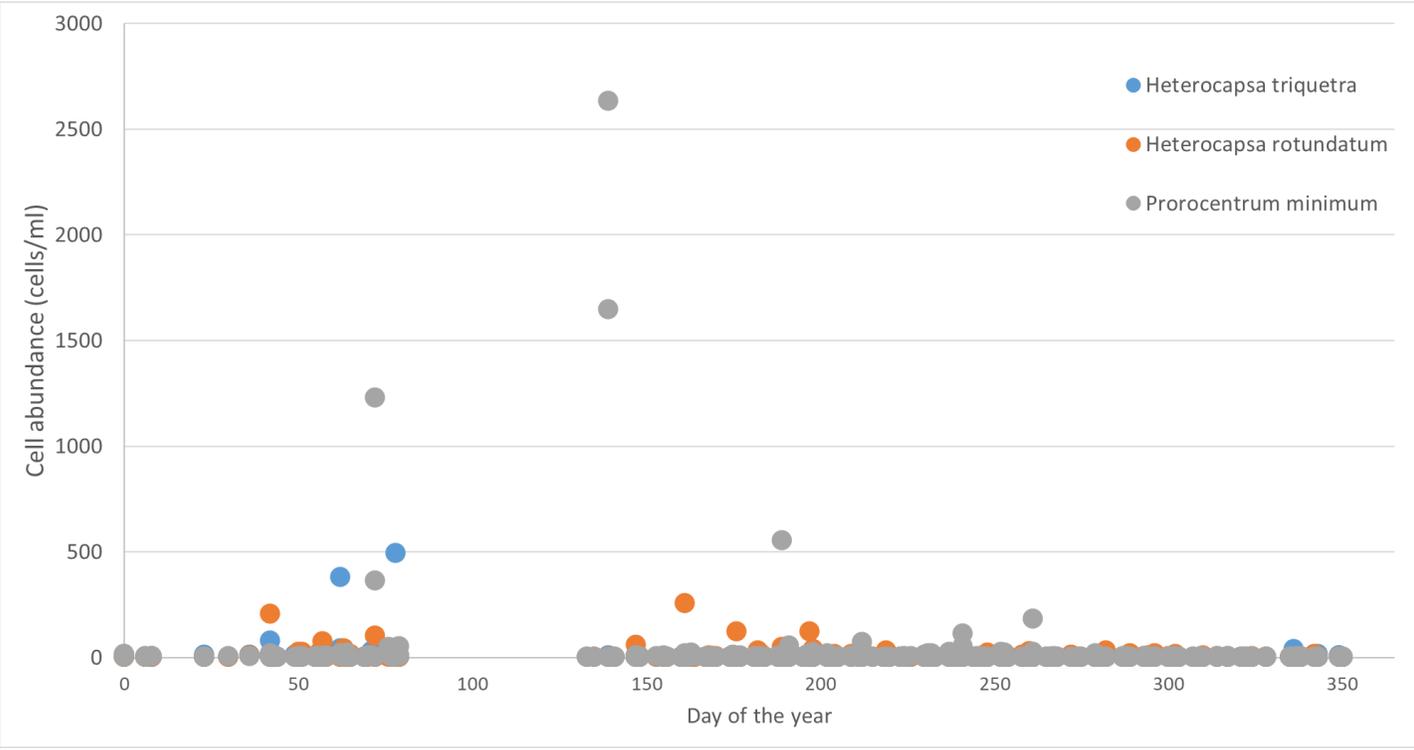


*Removed two *H. triquetra* values > 3000 cells/ml

Prorocentrum minimum

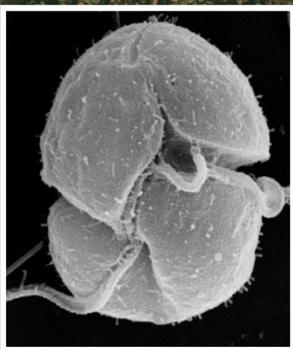


Bloom March – May (under-sampled due to COVID)

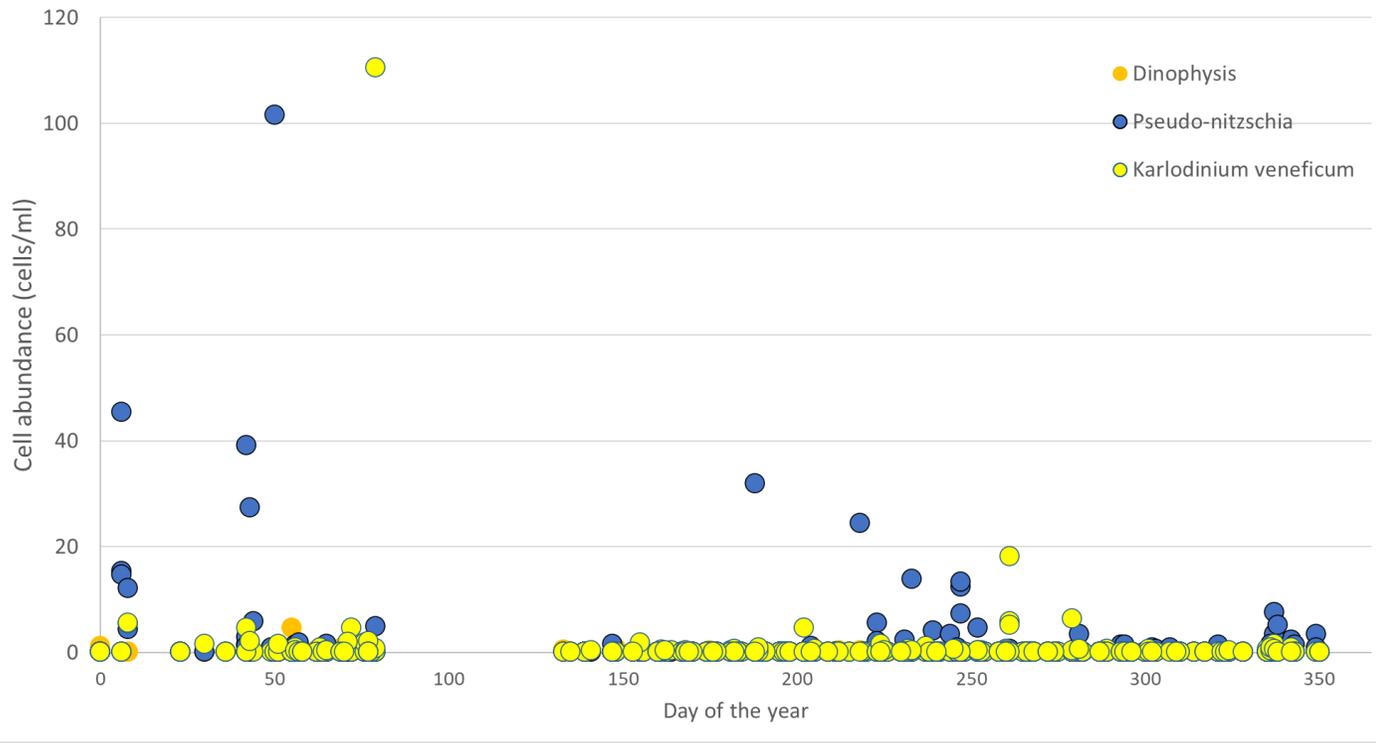


*Removed two *H. triquetra* values > 3000 cells/ml

Karlodinium veneficum



Low abundances but there was a gap in sampling due to COVID



2020HABs
K_veneficum

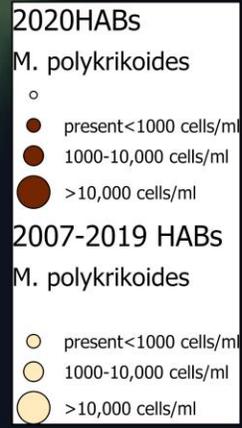
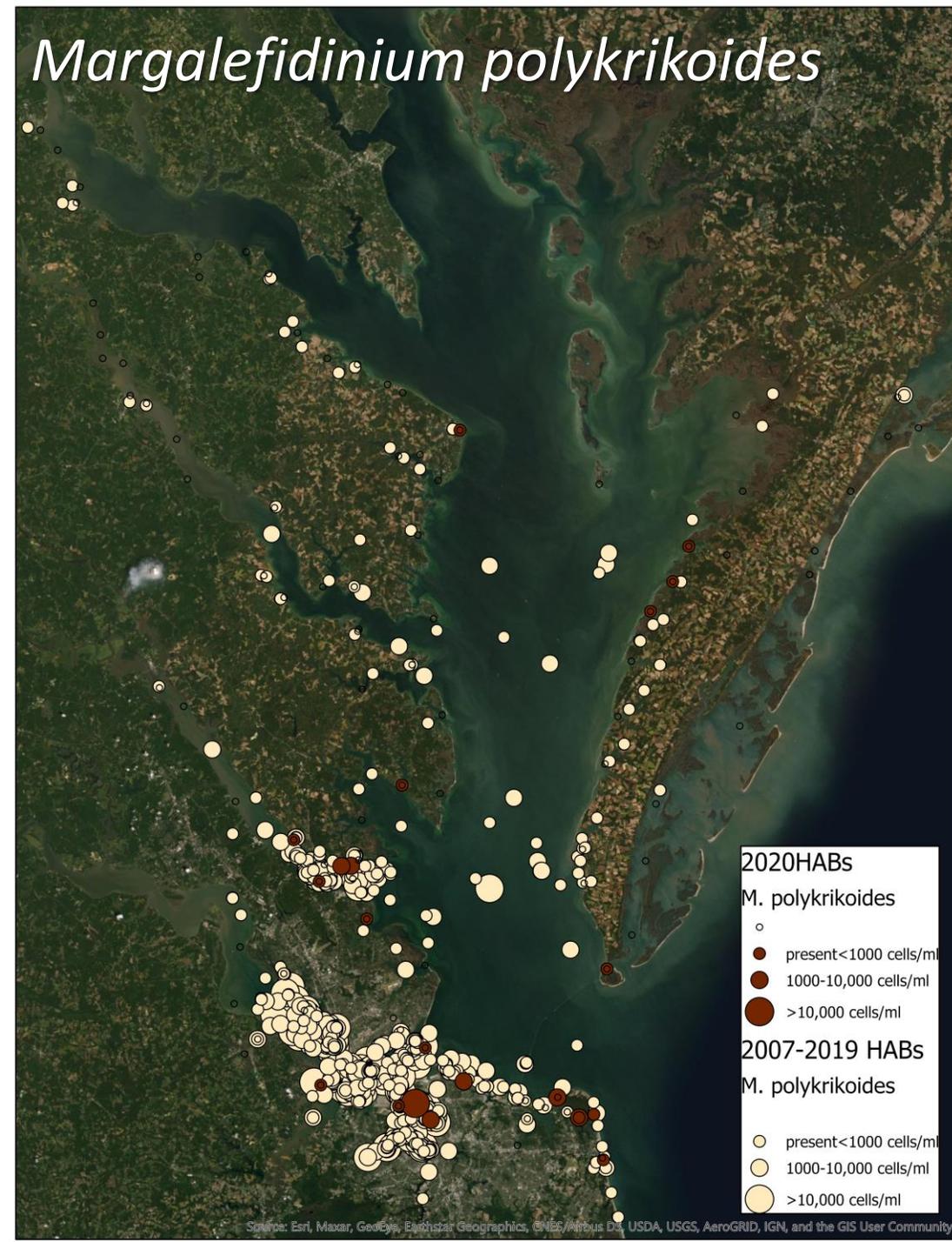
- present <500 cells/ml
- 500-3000 cells/ml
- >3000 cells/ml

2007-2019 HABs
K_veneficum

- present <500 cells/ml
- 500-3000 cells/ml
- >3000 cells/ml

Source: Esri, Maxar, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

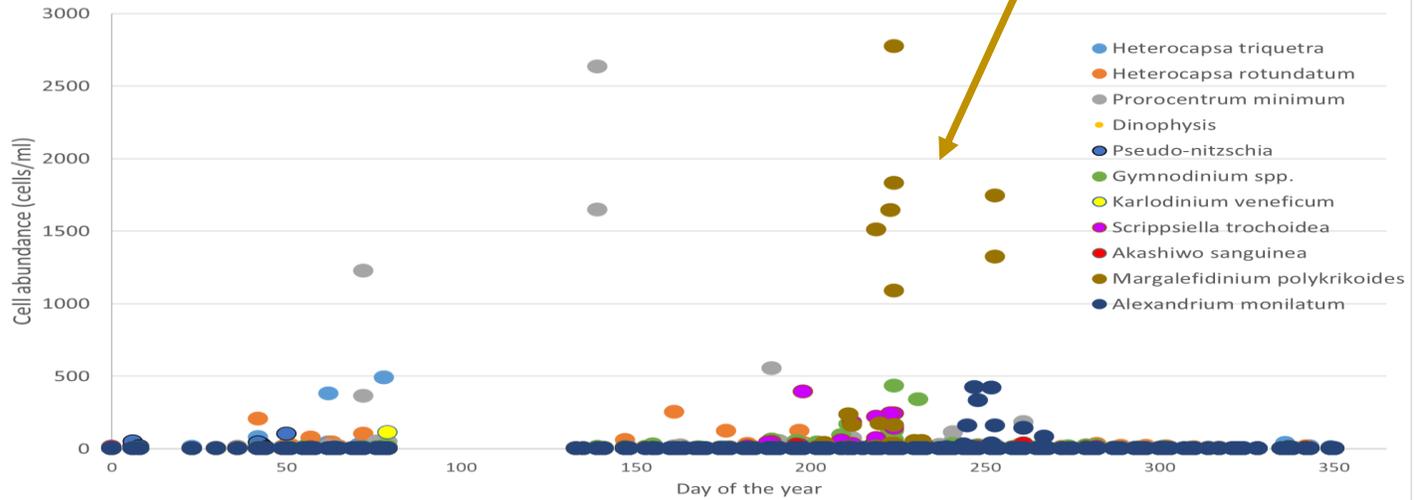
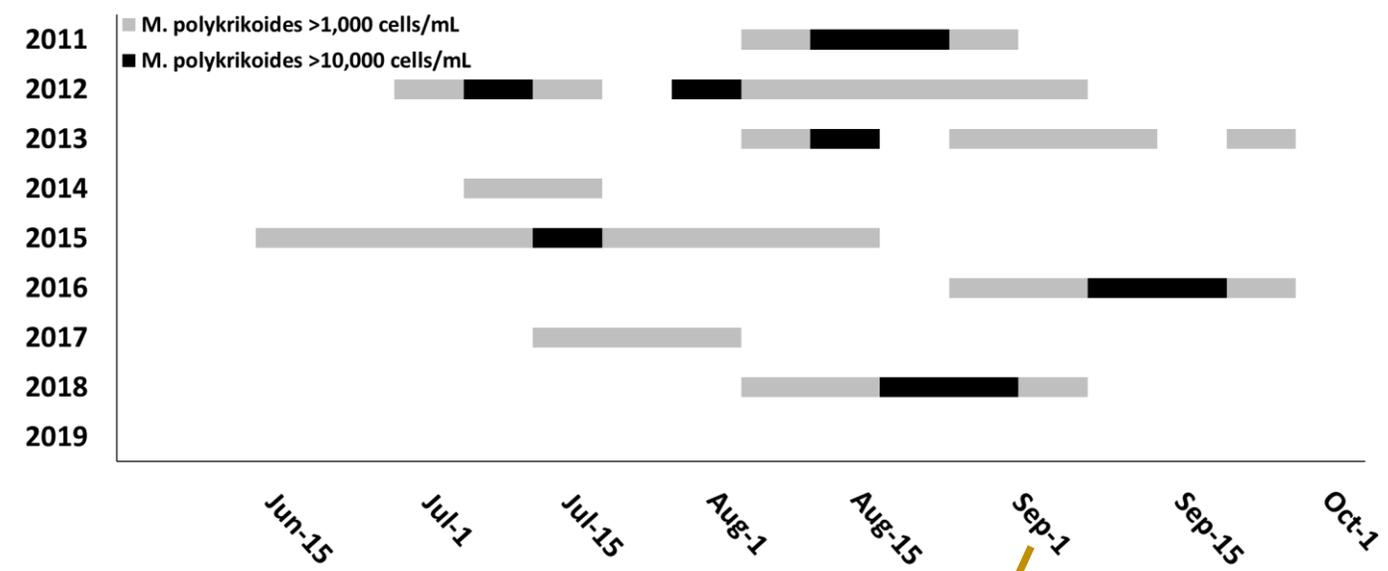
Margalefidinium polykrikoides



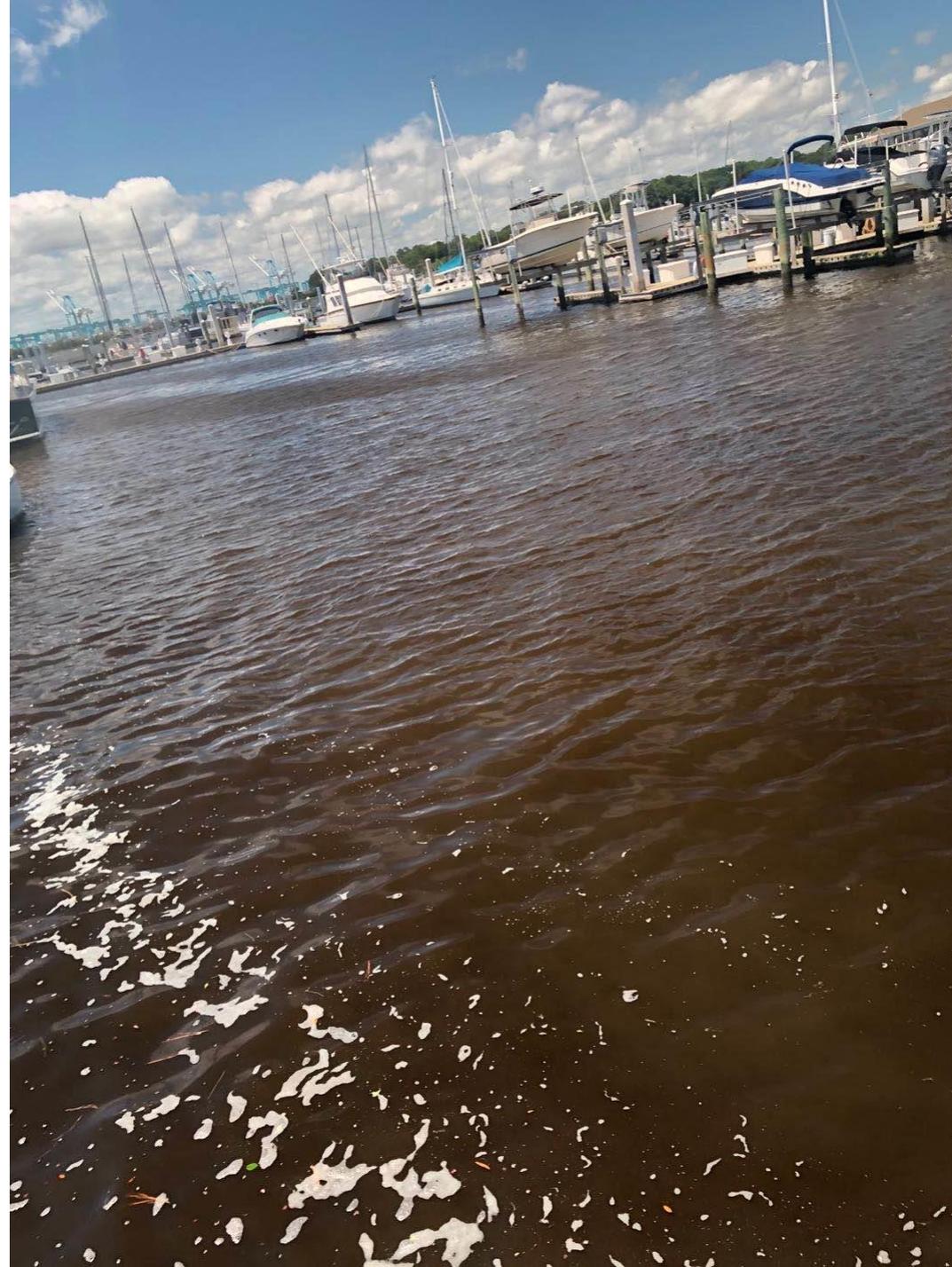
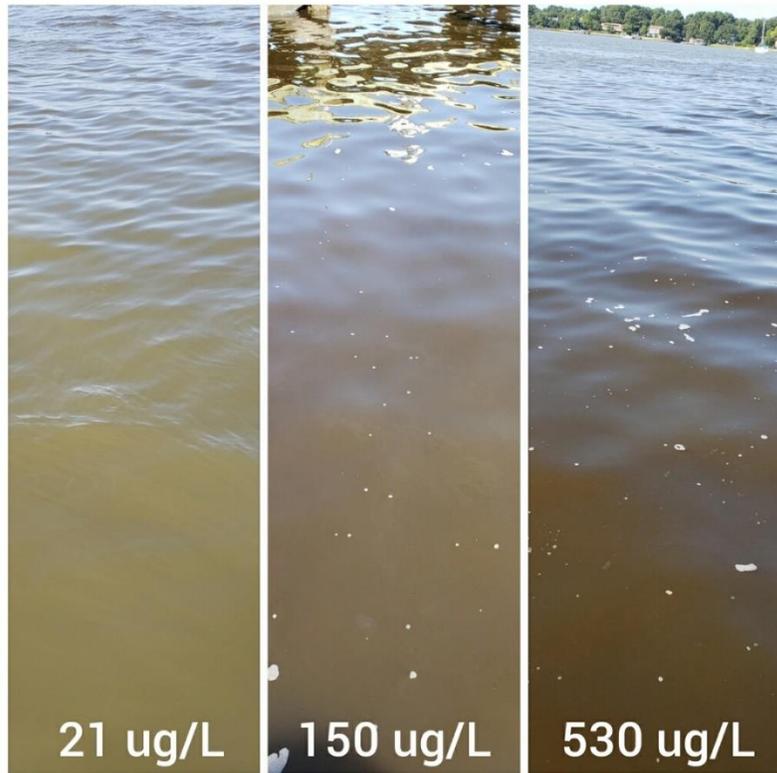
Source: Esri, Maxar, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

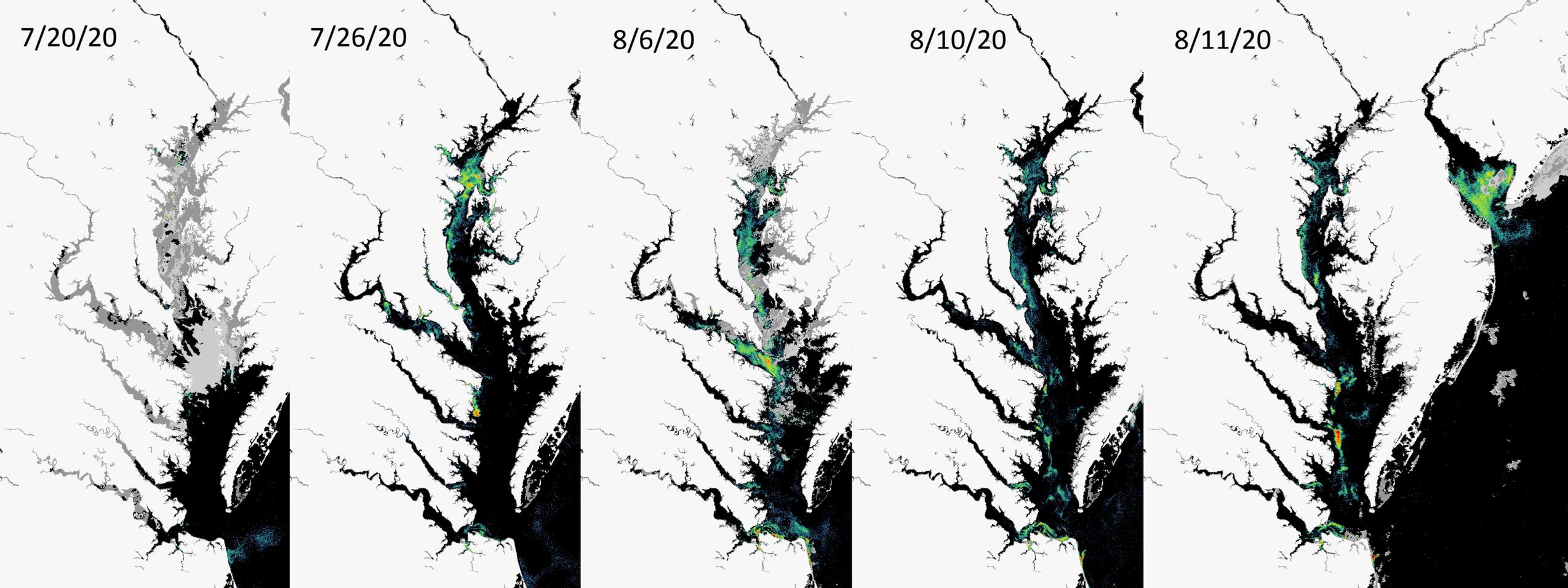
Margalefidinium polykrikoides

- Massive 2020 bloom – still counting time series samples
- Initiated in Lafayette River July 20, 2020 & reached oceanfront a week later
- Blooms in most of the last 15 – 20 years (not in 2019).

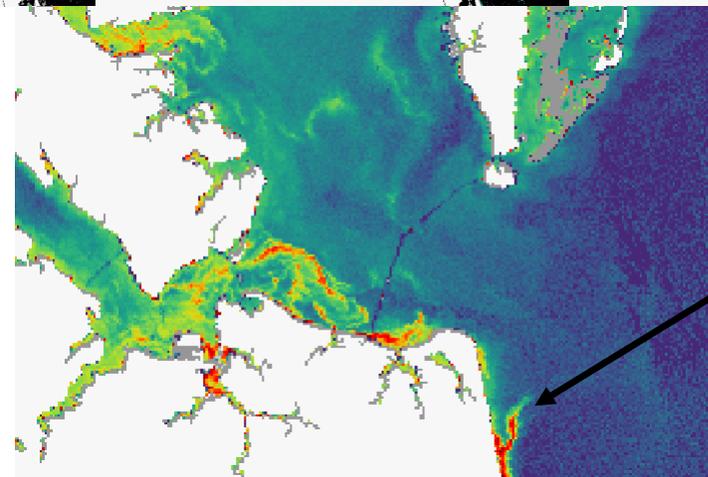


Chlorophyll concentrations in excess of 500 ug/L observed during the day.



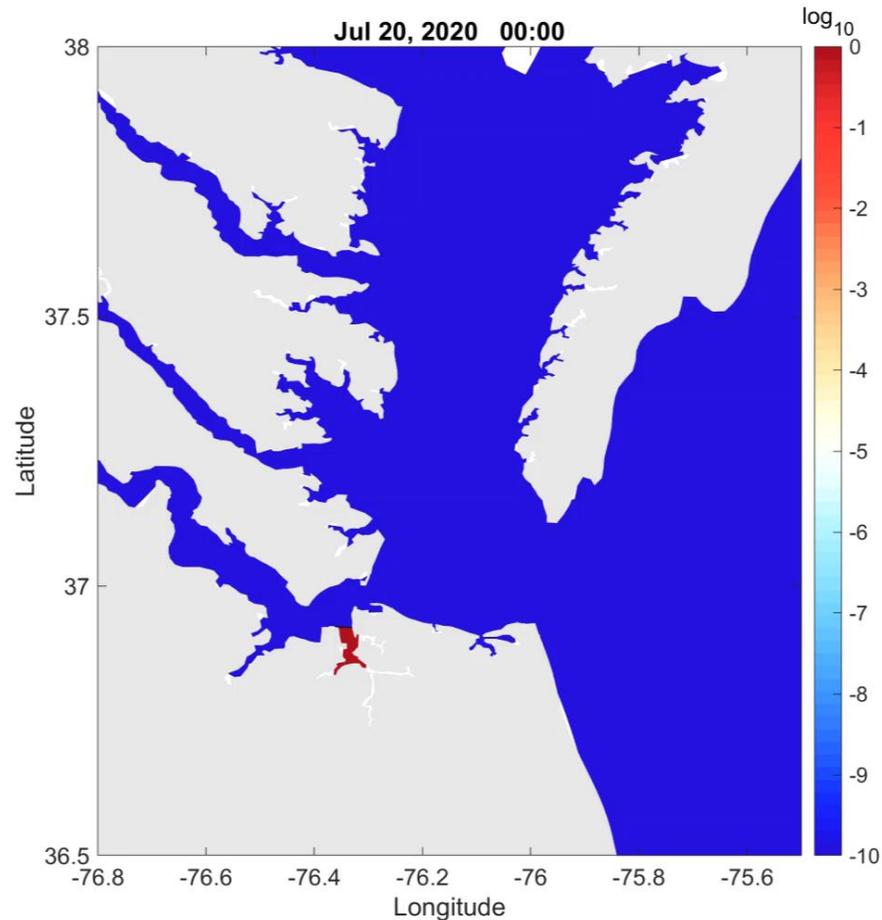


NOAA products (Shelly will tell you more this afternoon)



Observed at ocean front
down to NC

2020 Transport – collaboration with Qin & Shen



Dye study

Used calibrated and verified EFDC model for the Ches Bay (Hong & Shen 2012; Du & Shen 2017) and set up model for 2020 using wind data from NOAA, tidal elevation data from 3 station and river discharge data from USGS.

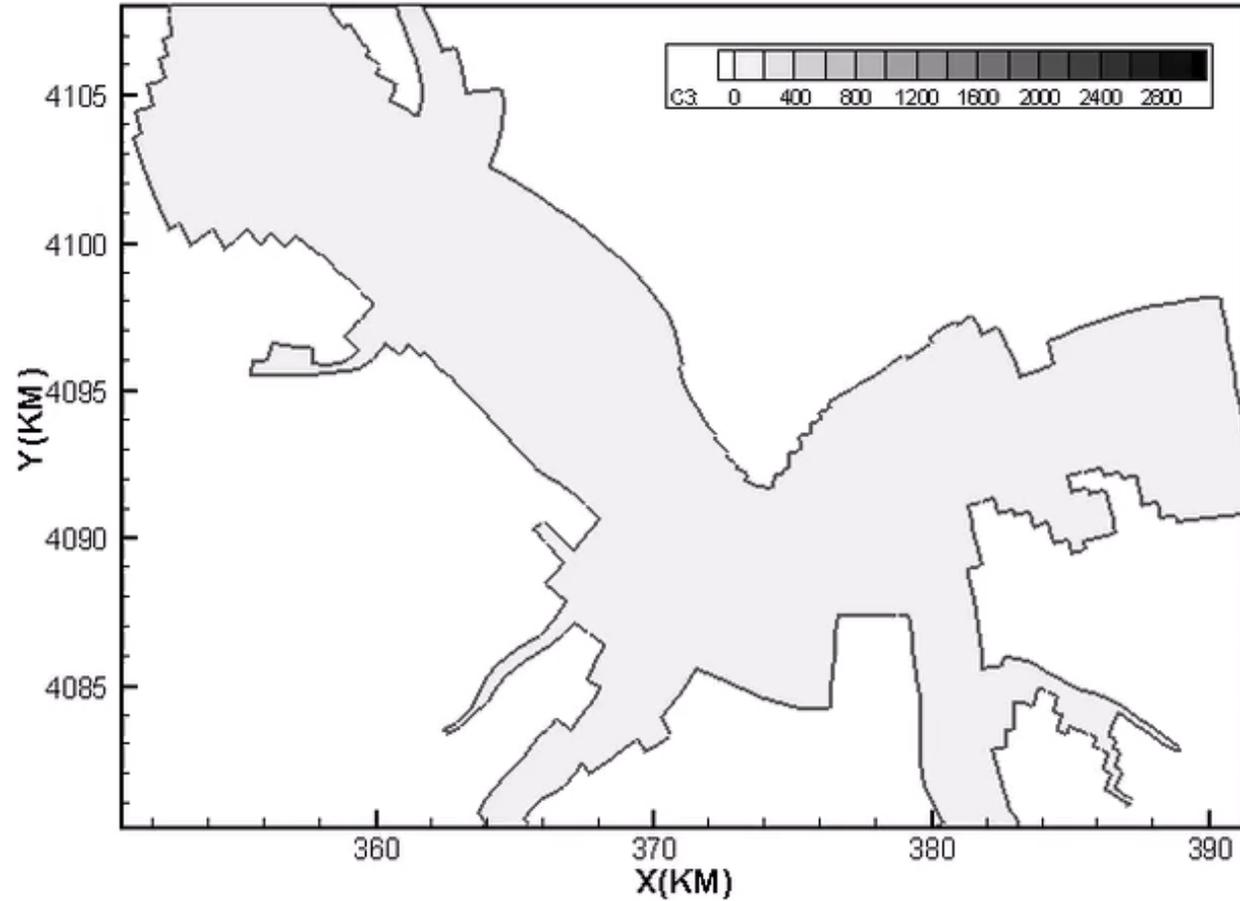
Dye predominantly travelled toward oceanfront, some propagated up Bay.

Big storm on August 4, 2020 which seemed to help transport the bloom.

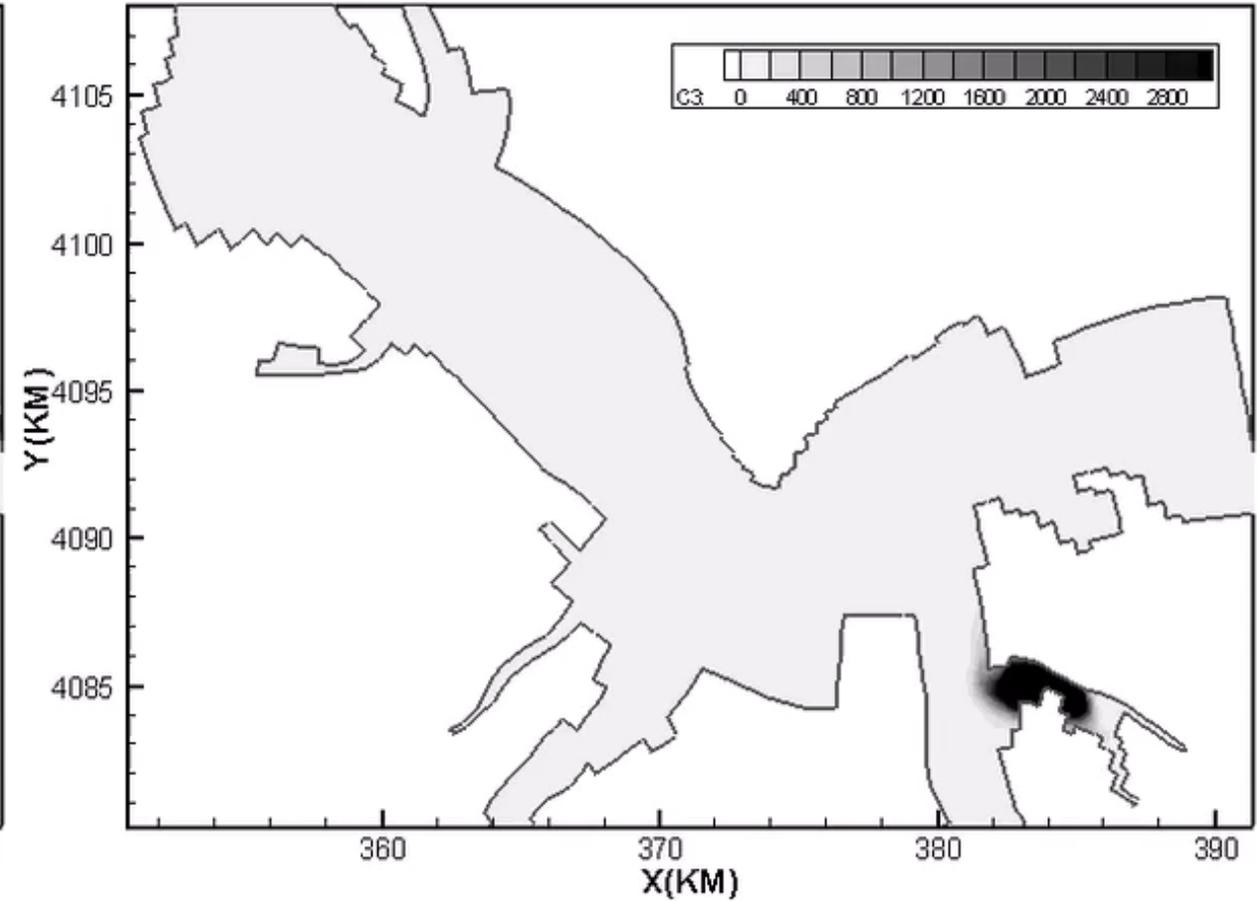
But bloom persisted.

2007 & 2008 Transport

ER Initiation 2007



Laf Initiation 2008

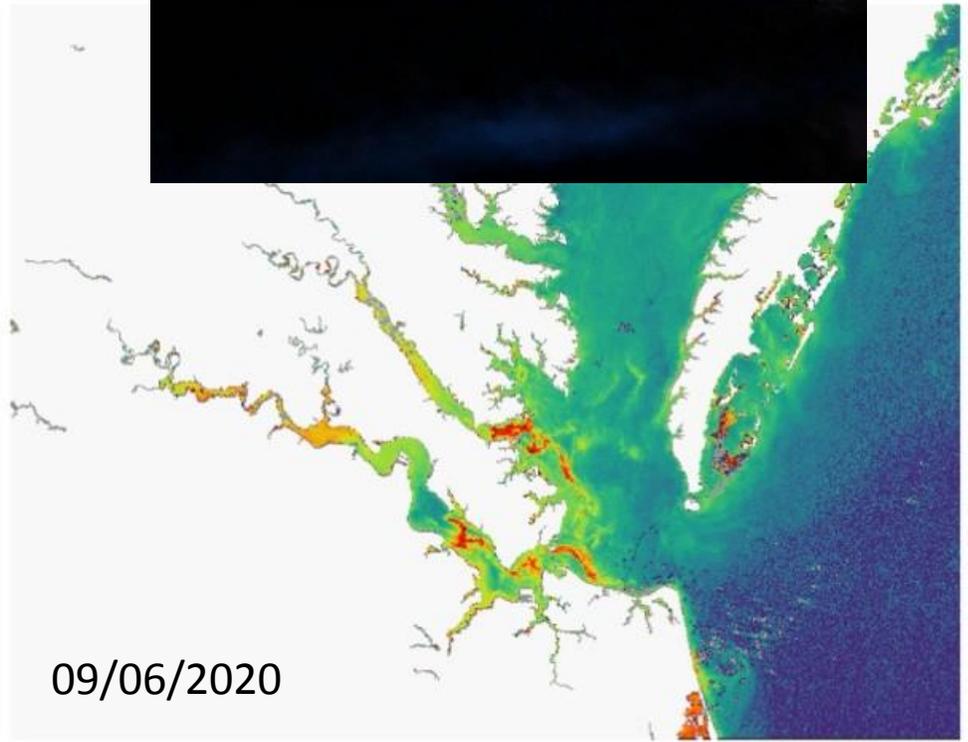
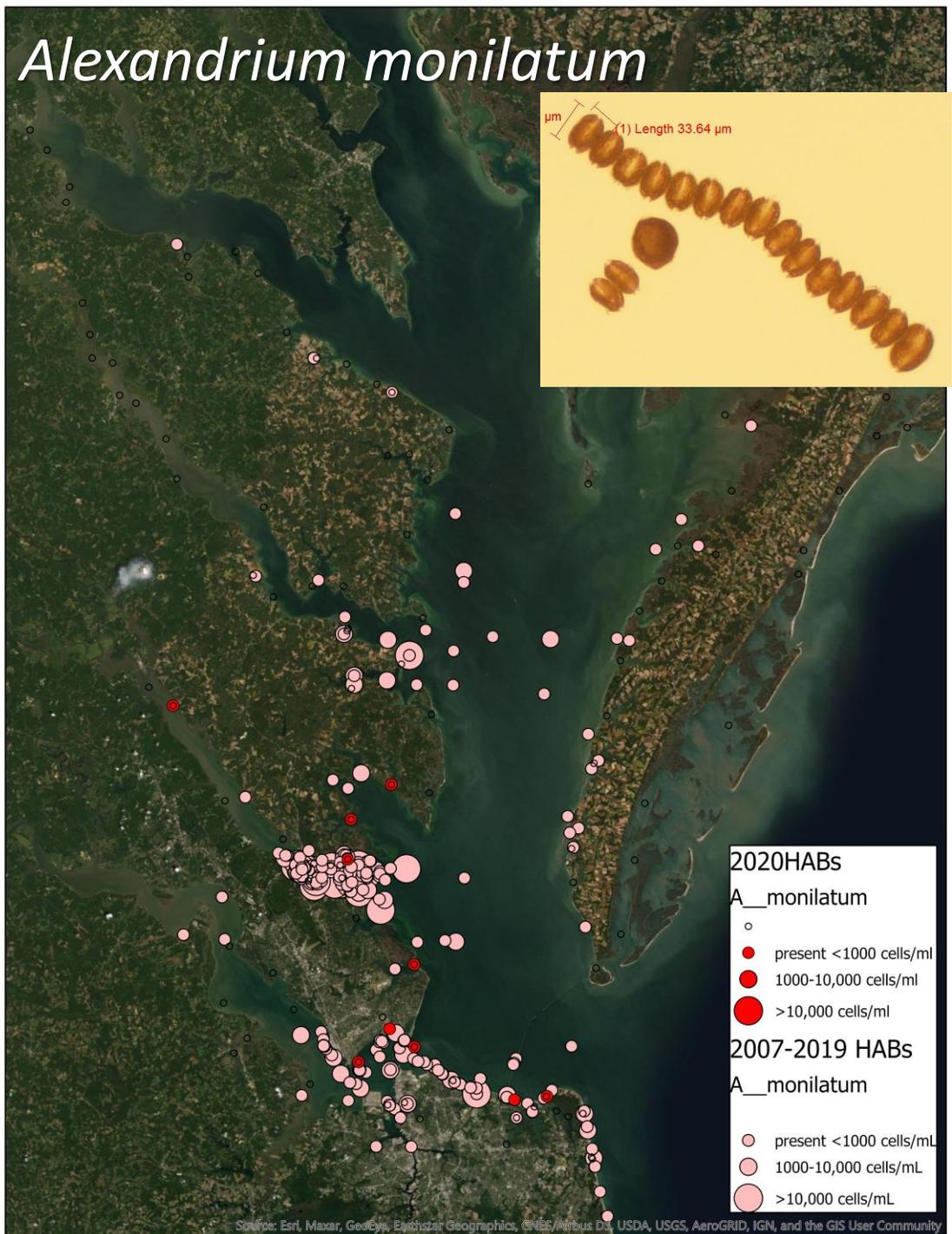


Alexandrium monilatum



Alexandrium monilatum – massive 2020 bloom

- *Alexandrium* – no bloom in 2018 or 2019
- Started in the York River and transported to Ocean View and ocean front



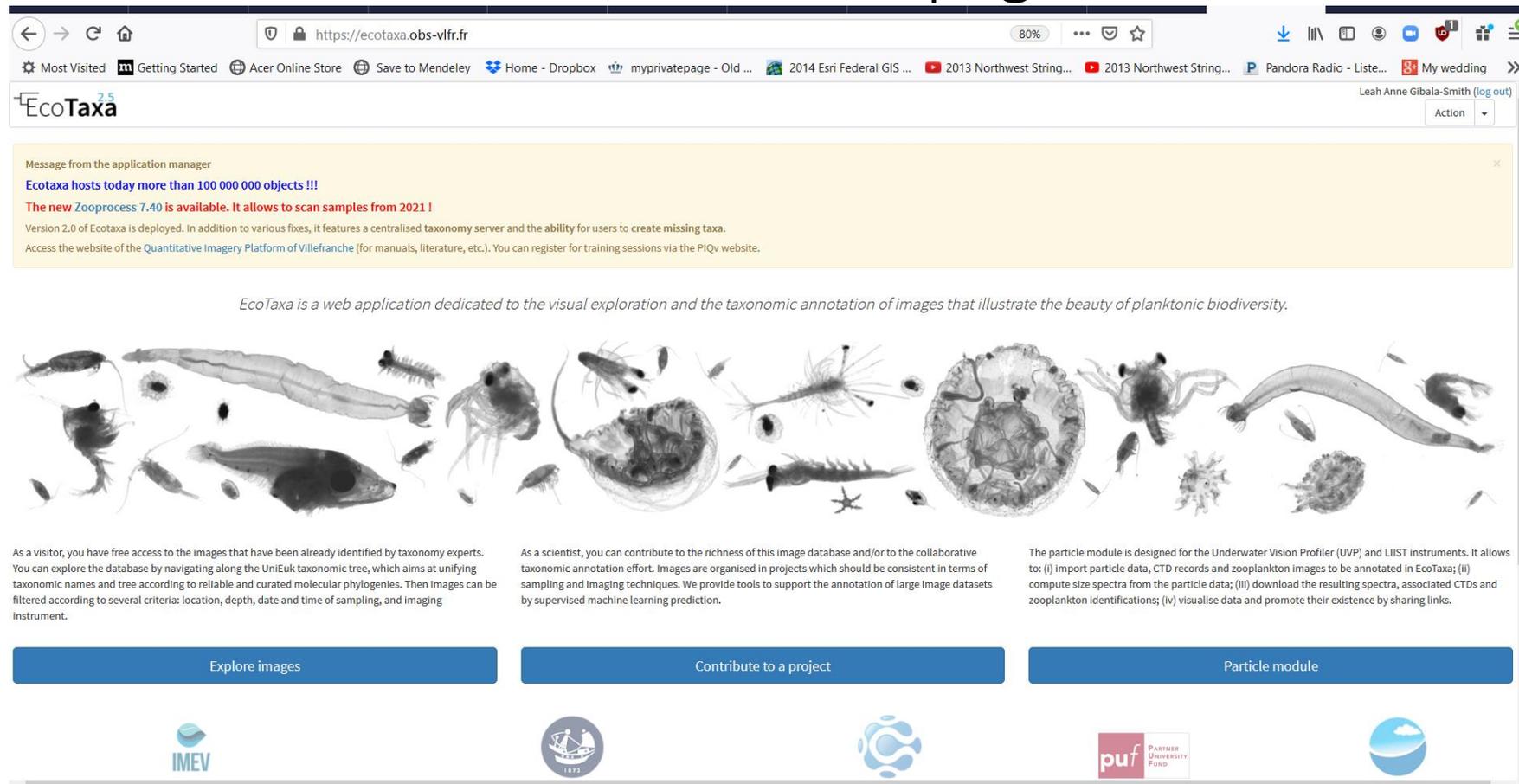
Source: Esri, Maxar, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

New opportunity in 2020 – what we started during COVID

IFCB & EcoTaxa

- Imaging flow cytobot (IFCB) images everything, even detritus, in a 5 mL sample (this was just what we fed it)
- Sophie Clayton loads images into EcoTaxa
- Leah and Kathryn (plus 2 undergraduate students) identify taxa and “train” software to identify taxa.

EcoTaxa homepage



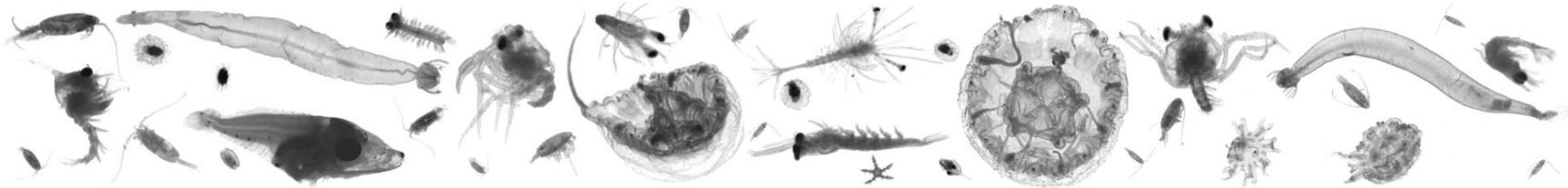
← → ↻ 🏠 <https://ecotaxa.obs-vlfr.fr> 80% ... 📧 ⭐

⚙️ Most Visited 📄 Getting Started 🌐 Acer Online Store 🌐 Save to Mendeley 📁 Home - Dropbox 👑 myprivatepage - Old ... 🗺️ 2014 Esri Federal GIS ... 📺 2013 Northwest String... 📺 2013 Northwest String... 📻 Pandora Radio - Liste... 📅 My wedding >>

EcoTaxa 2.5 Leah Anne Gibala-Smith (log out) Action ▾

Message from the application manager
EcoTaxa hosts today more than 100 000 000 objects !!!
The new Zooprocess 7.40 is available. It allows to scan samples from 2021 !
Version 2.0 of Ecotaxa is deployed. In addition to various fixes, it features a centralised taxonomy server and the ability for users to create missing taxa.
Access the website of the [Quantitative Imagery Platform of Villefranche](#) (for manuals, literature, etc.). You can register for training sessions via the PIQv website.

EcoTaxa is a web application dedicated to the visual exploration and the taxonomic annotation of images that illustrate the beauty of planktonic biodiversity.



As a visitor, you have free access to the images that have been already identified by taxonomy experts. You can explore the database by navigating along the UniEuk taxonomic tree, which aims at unifying taxonomic names and tree according to reliable and curated molecular phylogenies. Then images can be filtered according to several criteria: location, depth, date and time of sampling, and imaging instrument.

As a scientist, you can contribute to the richness of this image database and/or to the collaborative taxonomic annotation effort. Images are organised in projects which should be consistent in terms of sampling and imaging techniques. We provide tools to support the annotation of large image datasets by supervised machine learning prediction.

The particle module is designed for the Underwater Vision Profiler (UVP) and LIIST instruments. It allows to: (i) import particle data, CTD records and zooplankton images to be annotated in EcoTaxa; (ii) compute size spectra from the particle data; (iii) download the resulting spectra, associated CTDs and zooplankton identifications; (iv) visualise data and promote their existence by sharing links.

Explore images Contribute to a project Particle module

IMEV 1875 PUF PARTNER UNIVERSITY FUND

IFCB & EcoTaxa

- Images everything, even detritus, in a 5 mL sample
- We load images into EcoTaxa
- Leah and Kathryn (plus 2 undergraduate students) identify taxa and “train” software to identify taxa.

The screenshot displays the EcoTaxa 2.5 web interface. At the top, the logo "EcoTaxa 2.5" and the user name "LAPHER" are visible, along with a "Not logged (log in / register)" link. Below the header is a navigation bar with "Update view & apply filter", a "Select all" dropdown, and various icons for display, status, fit, zoom, and refresh. The main content area is a grid of microscopic images, each with a 10 µm scale bar and a green label indicating its taxonomic classification. The labels include terms like "small < detritus", "pennate 3 temp", "Chaetoceros chain", "Prorocentrum micans", "Heterocapsa triquetra", and "Cyanophyceae". On the left side, there is a "Taxonomy filter" section with a dropdown menu and a list of taxonomic groups with their respective counts. The groups listed include Alveolata (6), Amphidinium carterae (1), Amphidinium sp. (66), Amylax (91), Ciliophora (777), Mesodinium (179), Strombidium (136), Tintinnida (437), Tintinnopsis (19), Dinophysiaceae (40), Dinophysis (2), Diplopsalid group (1), Gonyaulax spinifera (6), Gymnodinium 01 (2), Gymnodinium 02 (298), Gymnodinium 03 (1328), Gyrodinium sp. (16), Gyrodinium (14), Heterocapsa (82), Heterocapsa rotundata (25822), Heterocapsa triquetra (28136), Heterocapsa 01 (79041), Heterocapsa 02 (1321), Heterocapsa 03 (2875), Heterocapsa 04 (1065), Heterocapsa 05 (362), Heterocapsa 06 (110), and Heterocapsa 07 (1065). At the bottom of the grid, a message states "Page management not available on FIT mode".

This is an unclassified upload of images from IFCB into ECOTAXA project ready for sorting and validating

The screenshot displays the EcoTaxa 2.5 web interface. At the top, the browser address bar shows the URL <https://ecotaxa.obs-vlfr.fr/prj/3800>. The page title is "LAPHTER_clean (0, 0, 0, 652106 / 652106)". The user is identified as "Leah Anne Gibala-Smith (log out)".

The interface features a navigation bar with "Project" and "Filtered" dropdowns, and a filter set to "Status=Unclassified". Below this is a control bar with "Update view & apply filter", "Select all", "Display", "Status", "Unclassified", "1000", "10", and "1 Selected".

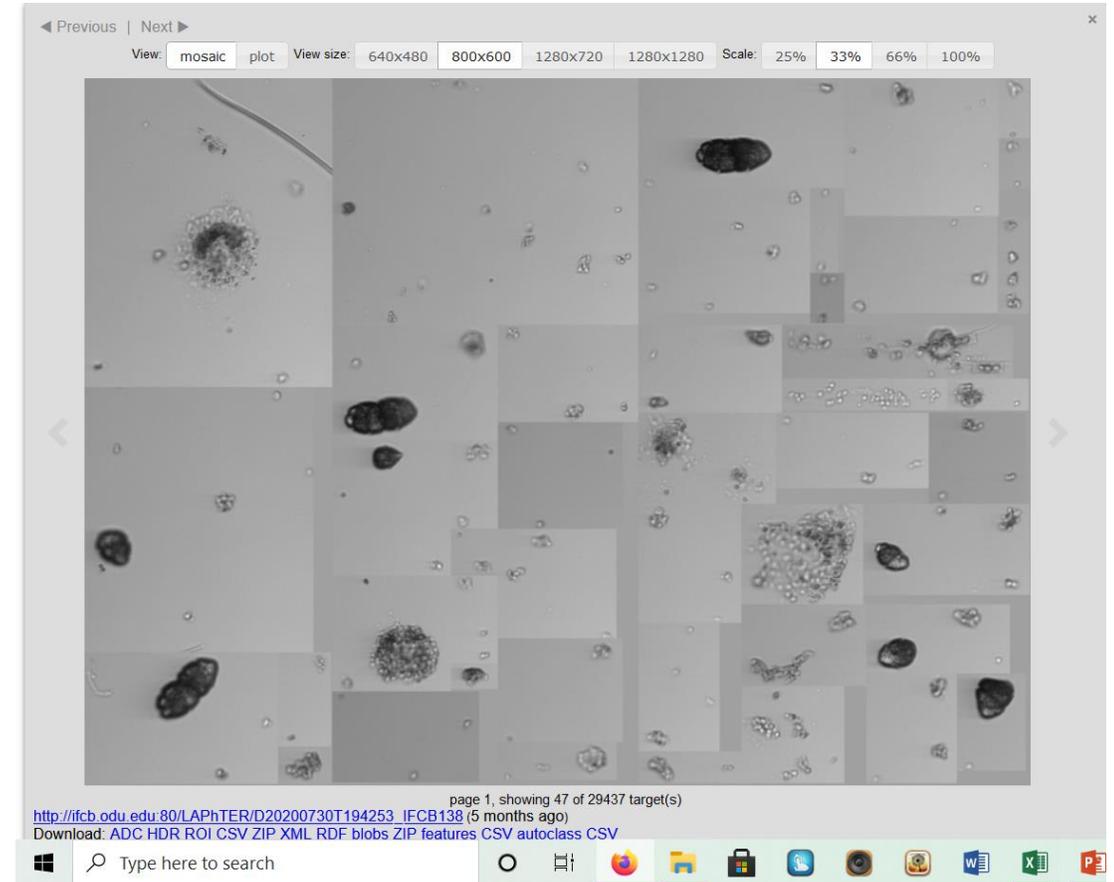
On the left, a "Taxonomy filter" sidebar lists various taxonomic groups with their respective counts:

- Achnanthes sp. 3
- Alveolata 1
- Alexandrium 1
- Ciliophora 10
 - Didinium 2
 - Mesodinium 40
 - Strombidium 17
 - Tintinnida 58
 - Tintinnopsis 2
- Dinophysis sp. 3
- Gymnodiniales 15
 - Akashiwo sanguinea < Akashiwo 29
 - Amphidinium sp. < Amphidinium 1
 - Gyrodinium 1
 - Polykrikos sp. 1
 - Warnowiaceae < Gymnodiniales 10
 - Heterocapsa rotundata 15

The main area contains a grid of microscopy images of microorganisms, each with a 10 µm scale bar. The Windows taskbar at the bottom shows the time as 8:26 AM on 1/11/2021.

Steps in procedure

1. Upload images and metadata from IFCB into EcoTaxa
2. Manually classify organisms – requires creation of new categories within taxonomic tree
3. Run predictive model on unclassified images to group them into taxa categories
4. Clean predictions by manually validating a portion of the correctly sorted cells and and moving incorrectly sorted cells into their correct taxonomic group
5. Run prediction on unvalidated sorted cells and manually validate/correct a portion of them
6. REPEAT



Garbage vs cells!!!

Prediction run – Only 1 cell predicted is a *P. minimum*

The screenshot shows the EcoTaxa 2.5 interface for a prediction run. The top bar includes the project name 'LAPhTER (0, 6983, 0, 0 / 6983)' and the user 'Leah Anne Gibala-Smith (log out)'. The filter is set to 'Taxo= Prorocentrum minimum' and 'Status= Predicted'. The main area displays a grid of 90 cells, each with a micrograph and a prediction label. The labels are mostly 'Prorocentrum minimum' with taxonomic details below. A single cell in the bottom row, eighth column, is circled in pink, indicating it is the only one predicted as a *P. minimum*.

Cell Index	Micrograph	Prediction Label
1-9	[Micrographs]	Prorocentrum minimum < Prorocentrum < Prorocentraceae
10-18	[Micrographs]	Prorocentrum minimum < Prorocentrum < Prorocentraceae
19-27	[Micrographs]	Prorocentrum minimum < Prorocentrum < Prorocentraceae
28-36	[Micrographs]	Prorocentrum minimum < Prorocentrum < Prorocentraceae
37-45	[Micrographs]	Prorocentrum minimum < Prorocentrum < Prorocentraceae
46-54	[Micrographs]	Prorocentrum minimum < Prorocentrum < Prorocentraceae
55-63	[Micrographs]	Prorocentrum minimum < Prorocentrum < Prorocentraceae
64-72	[Micrographs]	Prorocentrum minimum < Prorocentrum < Prorocentraceae
73-81	[Micrographs]	Prorocentrum minimum < Prorocentrum < Prorocentraceae
82-90	[Micrographs]	Prorocentrum minimum < Prorocentrum < Prorocentraceae

Validated and corrected

The screenshot displays the EcoTaxa 2.5 web interface. At the top, the project name 'LAPhTER (4923, 0, 0, 0 / 4923)' is shown. The filter bar indicates 'Taxo= Prorocentrum minimum' and 'Status= Validated'. The user 'Leah Anne Gibala-Smith' is logged in. The interface includes a 'Select all' button, a 'Display' dropdown, and a 'Status' dropdown set to 'Validated'. The main content area shows a grid of 90 organism images, each with a 10 µm scale bar. The taxonomic classification for each image is displayed below it, mostly as 'Prorocentrum minimum' and '< Prorocentrum' under the family 'Prorocentraceae'. On the left, a 'Taxonomy filter' sidebar lists various taxonomic levels with counts: Prorocentrum 01 (4), Prorocentrum micans (40), Prorocentrum minimum (4923), Scrippsiella sp. (15), Warnowiaceae (98), dinoflagellate-i (6), dinoflagellate-ii (8), dinoflagellate-iii (76), dinoflagellate-iv (81), dinoflagellate-ix (50), dinoflagellate-v (10), dinoflagellate-vi (119), dinoflagellate-vii (2), dinoflagellate-viii (49), dinoflagellate-x (1207), dinoflagellate-xi (4), and Arthropoda (13).

There are inconsistencies in model predictions for some organisms because cell morphology can be highly variable. So, make sub-categories to account for life stages, etc. - more on this later – and rerun the model, it is an iterative process.

Eventually, we can group cells and train the instrument to count them in individual samples

Akashiwo

The screenshot displays the EcoTaxa 2.5 web interface. At the top, the project name is "LAPhTER_clean (29, 0, 0, 0 / 29)". The filter is set to "Taxo= Akashiwo sanguinea" and "Status= Validated". The user is identified as Leah Anne Gibala-Smith. The interface shows a grid of 18 microscopy images of Akashiwo sanguinea cells, arranged in 3 rows and 6 columns. Each image includes a 10 µm scale bar and a taxonomic label: "Akashiwo sanguinea < Akashiwo < Gymnodiniaceae < Gymnodiniales". On the left, a taxonomy filter is visible, listing various groups with their counts: Achnanthes sp. (3), Alveolata (1), Alexandrium (1), Ciliophora (10), Didinium (2), Mesodinium (40), Strombidium (17), Tintinnida (58), Tintinnopsis (2), Dinophysis sp. (3), Gymnodiniales (15), Akashiwo sanguinea < Akashiwo < Gymnodiniaceae < Gymnodiniales (29), Amphidinium sp. < Amphidinium (1), Gyrodinium (1), Polykrikos sp. (1), Warnowiaceae < Gymnodiniales (10), Heterocapsa rotundata (15), Heterocapsa triquetra < Heterocapsa (7), and Prorocentrum micans < Prorocentrum (36). The Windows taskbar at the bottom shows the time as 8:09 AM on 1/11/2021.

Dinophysis

The screenshot shows the EcoTaxa 2.5 interface. The project name is "LAPhTER_clean (8, 0, 0, 0/3)". The filter is set to "Taxo= Dinophysis sp." and "Status= Validated". The interface includes a "Taxonomy filter" on the left with a list of taxa: Achnanthes sp. (3), Alveolata (1), Alexandrium (1), Ciliophora (10), Didinium (2), Mesodinium (40), Strombidium (17), Tintinnida (58), Tintinnopsis (2), Dinophysis sp. (3), and Gymnodiniales (16). The main view displays three micrographs of Dinophysis sp. specimens, each with a 10 µm scale bar. Below the micrographs are buttons for "Save pending changes [CTRL+S]", "Validate all and move to next page", and "Validate Selection [CTRL+L]".

Even the rare ones! We can examine more sample in detail so this may help us!

Pseudo-nitzschia

The screenshot shows the EcoTaxa 2.5 interface for a different project, "LAPhTER (43, 0, 0, 0/43)". The filter is set to "Taxo= Pseudo-nitzschia sp." and "Status= Validated". The user is identified as "Leah Anne Gibala-Smith (log out)". The interface includes a "Taxonomy filter" on the left with a list of taxa: Bacillariophyceae (0), Achnanthes (9), Amphiprora (7792, 933), Bacillaria (2), Delphineis (2), Diatoma (0), Grammatophora (7), Tabellaria (103, 10), Asterionellopsis glacialis, Asterionellopsis (301, 14), Cocconeis sp. (141, 2), Cylindrotheca closterium, Cylindrotheca (4096, 308), Gomphonema sp. (11), Gyrosigma fasciola (18), Pseudo-nitzschia sp. (43, 16), Synedra sp. (6, 3), Thalassionema nitzschioides, Thalassionema (19), Campylosira (2), Cerataulina 2 temp (1), and Cerataulina pelagica (1948, 154). The main view displays a grid of 15 micrographs of Pseudo-nitzschia sp. specimens, each with a 10 µm scale bar. The interface includes buttons for "Update view & apply filter", "Select all", "Display", "Status", "Validated", "1000", "80%", "1 Selected", and "Action".

Heterocapsa triquetra – we're also learning about the organisms themselves!

EcoTaxa 2.5 Project Filtered LAPhTER (79036, 0, 0, 0 / 79036) Leah Anne Gibala-Smith (log out)

Filter: Taxo= Heterocapsa triquetra Status= Validated

Update view & apply filter Select all Display Status Validated 1000 50

Taxonomy filter Other filters

- Tintinnida 40
- Tintinnopsis 1
- Dinophysiaceae < 3
- Dinophysis 7
- Diplopsalid group 7
- Gonyaulax spinifera < Gonyaulax 2
- Gymnodinium 01 53
- Gymnodinium 02 282 1329
- Gymnodinium 03 82 18
- Gyrodinium sp. < Gyrodinium 127 14
- Heterocapsa 1**
 - Heterocapsa rotundata 24177 30002
 - Heterocapsa triquetra < Heterocapsa 79036 1427**
 - Heterocapsa 01 2703 1204
 - Heterocapsa triquetra < Heterocapsa 01 373 40
 - Heterocapsa 02 99 7
 - Heterocapsa 04 2292 9267
 - Karlodinium sp. < Karlodinium

Heterocapsa triquetra – we're seeing different life stages

Browser: <https://ecotaxa.obs-vlfr.fr/prj/2840> | 110% | Leah Anne Gibala-Smith (log out)

EcoTaxa 2.5 | Project | Filtered | Filter: Taxo= Heterocapsa triquetra | Status= Validated | Action

LAPhTER (373, 0, 0, 0 / 373)

Update view & apply filter | Select all | Display | Status: Validated | 1000 | 60%

Taxonomy filter | Other filters

- Dinophysis: 7
- Diplopsalid group: 7
- Gonyaulax spinifera < Gonyaulax: 2
- Gymnodinium 01: 53
- Gymnodinium 02: 282 | 1329
- Gymnodinium 03: 82 | 18
- Gyrodinium sp. < Gyrodinium: 127 | 14
- Heterocapsa: 1**
 - Heterocapsa rotundata: 24177 | 30002
 - Heterocapsa triquetra < Heterocapsa: 79036 | 1427
 - Heterocapsa 01: 2703 | 1204**
 - Heterocapsa triquetra < Heterocapsa 01: 373 | 40**
 - Heterocapsa 02: 99 | 7
 - Heterocapsa 04: 2292 | 9267
 - Karlodinium sp. < Karlodinium: 87 | 212
- < Dinophyceae X

Grid of 30 microscopy images showing various life stages of *Heterocapsa triquetra*. Each image includes a 10 µm scale bar and a caption such as "Heterocapsa triquetra < Heterocapsa 01". The images illustrate different developmental forms, including single cells, pairs, and larger, more complex structures.

Heterocapsa triquetra Peridinale

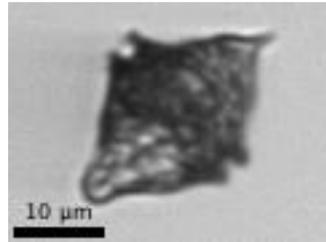
Asexual reproduction observed: Eleutheroschisis: theca sheds before or after cell division

Sexual reproduction not observed

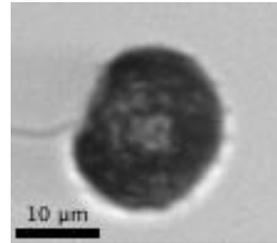
Heterocapsa groupings:

ECOTAXA CATEGORY TREE & definitions (intermediate working arrangement)

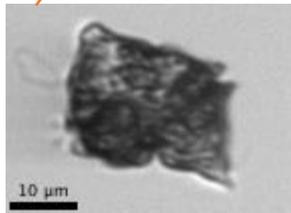
▼ Heterocapsa	1
Heterocapsa rotundata	23853 6601
Heterocapsa triquetra < Heterocapsa	72908 5762
▼ Heterocapsa 01	635 2116
Heterocapsa triquetra < Heterocapsa 01	287 37
Heterocapsa 02	33 8
Heterocapsa 04	2085 9182



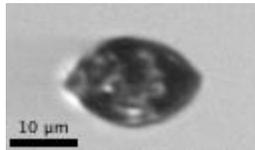
Heterocapsa triquetra
Vegetative cell



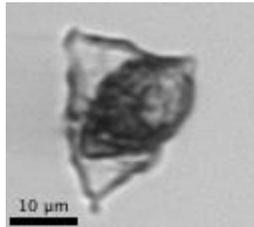
Heterocapsa 04
antapical view of
the vegetative cell



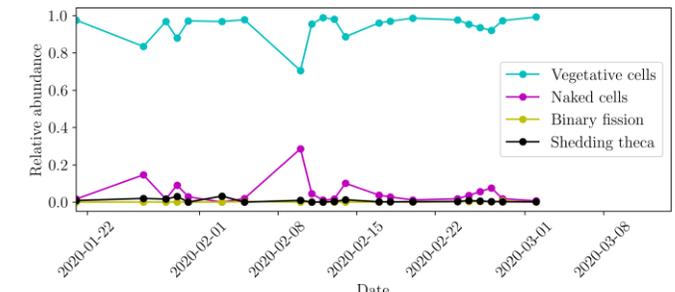
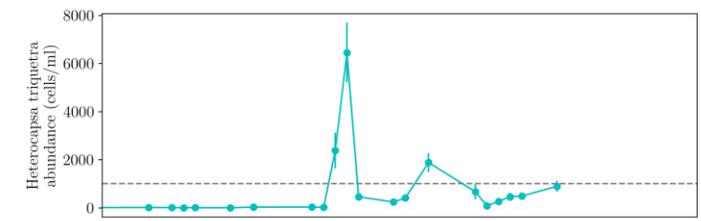
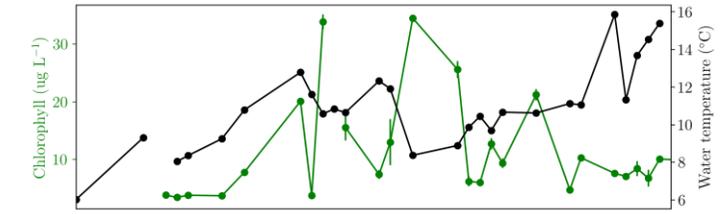
Heterocapsa 02
Binary fission



Heterocapsa 01
Ecdysis has occurred -"naked" cells that have shed their theca (part of asexual reproduction) OR can be the result of stress



Heterocapsa triquetra < Heterocapsa 01
Cells in the process of shedding their theca but still have an intact pellicle



IFCB live dashboard – *Margalefidinium* bloom – images not uploaded to Ecotaxa yet
We know life stages and chain length may play an important role in the initiation and persistence of these blooms and plan to interrogate the database.

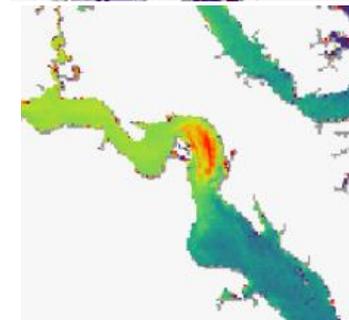
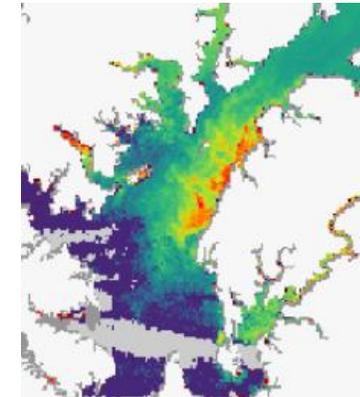


2021 program plans

- Continue VDH & CBP sampling and merging of databases
- Resume HRSD dataflow sampling after COVID hiatus
- Continue sampling at the Lafayette River time series
- Merging of databases
- Continue IFCB training
 - Also getting Flow Cam loaner this winter
- Building off multiple programs – VDH, CBP, HRSD, ECOHAB
- Continued discussions with partners
 - Phytoplankton methods- IFCB, remote sensing
 - Management strategies

New year's resolutions/goals

- Find a way to get a dedicated IFCB
- Year-round sampling and analyses with undergrads and instruments
- Laboratory experiments
 - Isolation of cultures
 - Production of toxins
 - Life cycle events that influence blooms
- More student involvement
- New funding to tie in research with the monitoring to better advise management



Thank you!

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People:

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Questions?

